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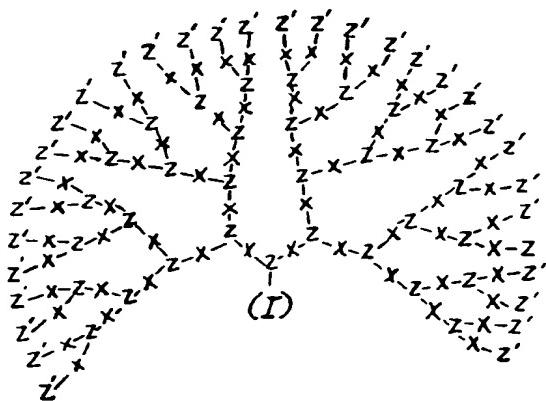
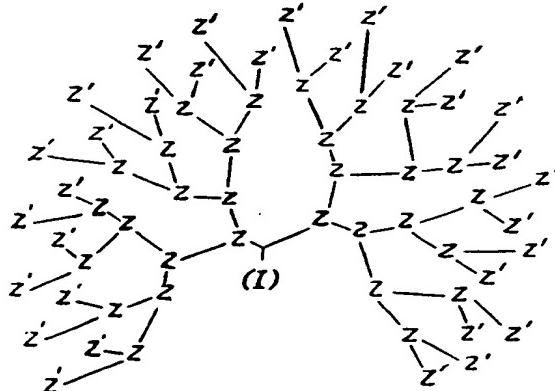
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(54) Title: STARBURST CONJUGATES



(57) Abstract

Starburst conjugates which are composed of at least one dendrimer in association with at least one unit of a carried agricultural material have been prepared. These conjugates have particularly advantageous properties due to the unique characteristics of the dendrimer.

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STARBURST CONJUGATES

The present invention concerns the use of dense star polymers as carriers for agricultural materials. In recent years polymers referred to as dense star polymers or starburst polymers have been developed. It has been found that the size, shape and properties of these dense star polymers or starburst polymers can be molecularly tailored to meet specialized end uses. Starburst polymers have significant advantages which can provide a means for the delivery of high concentrations of carried material per unit of polymer, controlled delivery, targeted delivery and/or multiple species delivery or use.

In its broadest aspect, the present invention is directed to polymer conjugate materials comprising dense star polymers or starburst polymers associated with desired agricultural materials (hereinafter these polymer conjugates will frequently be referred to as "starburst conjugates" or "conjugates"), processes for preparing these conjugates, compositions

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containing the conjugates, and methods of using the conjugates and compositions.

The conjugates of the present invention are suitable for use in a variety of applications where specific delivery is desired, and are particularly suited for the delivery of biologically active agents. In a preferred embodiment of the present invention, the starburst conjugates are comprised of one or more starburst polymers associated with one or more bioactive agents.

The starburst conjugates offer significant benefits over other carriers known in the art due to the advantageous properties of the starburst polymers. Starburst polymers exhibit molecular architecture characterized by regular dendritic branching with radial symmetry. These radially symmetrical molecules are referred to as possessing "starburst topology". These polymers are made in a manner which can provide concentric dendritic tiers around an initiator core. The starburst topology is achieved by the ordered assembly of organic repeating units in concentric, dendritic tiers around an initiator core; this is accomplished by introducing multiplicity and self-replication (within each tier) in a geometrically progressive fashion through a number of molecular generations. The resulting highly functionalized molecules generations have been termed "dendrimers" in deference to their branched (tree-like) structure as well as their oligomeric nature. Thus, the terms starburst oligomer and starburst dendrimer are encompassed within the term starburst polymer. Topological polymers, with size and shape controlled domains, are dendrimers that are covalently bridged

through their reactive terminal group which are referred to as "starburst bridged dendrimers", which is also encompassed within the term starburst polymer.

5 The following description of the figures aids in understanding the present invention.

Figure 1 depicts various generations of starburst dendrimers.

10 Figure 2A depicts a dendrimer having unsymmetrical (unequal) branch junctures.

Figure 2B depicts a dendrimer having symmetrical (equal) branch junctures.

15 Figure 3 shows carbon-13 spin lattice relaxation times (T_1) for aspirin incorporated into various dendrimer generations. (Example 4)

20 The starburst polymers are illustrated by Figure 1 wherein I represents an initiator core (in this figure a tri-functional initiator core, shown by the far left drawing); Z represents a terminal group, shown in the first instance by the second drawing from the left, referred to as a starbranched oligomer; A, B, C, D, and E represent particular molecular generations of starburst oligomers, called dendrimers; and $(A)_n$, $(B)_n$, $(C)_n$, $(D)_n$, and $(E)_n$ represent starburst bridged dendrimers.

25 30 35 The starburst dendrimers are unimolecular assemblages that possess three distinguishing architectural features, namely, (a) an initiator core, (b) interior layers (generations, G) composed of repeating units, radially attached to the initiator

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core, and (c) an exterior surface of terminal functionality (i.e., terminal functional groups) attached to the outermost generation. The size and shape of the starburst dendrimer molecule and the 5 functional groups present in the dendrimer molecule can be controlled by the choice of the initiator core, the number of generations (i.e., tiers) employed in creating the dendrimer, and the choice of the repeating units employed at each generation. Since the 10 dendrimers can be readily isolated at any particular generation, a means is provided for obtaining dendrimers having desired properties.

15 The choice of the starburst dendrimer components affects the properties of the dendrimers. The initiator core type can affect the dendrimer shape, producing (depending on the choice of initiator core), for example, spheroid-shaped dendrimers, cylindrical or 20 rod-shaped dendrimers, ellipsoid-shaped dendrimers, or mushroom-shaped dendrimers. Sequential building of generations (i.e., generation number and the size and nature of the repeating units) determines the dimensions of the dendrimers and the nature of their 25 interior.

Because starburst dendrimers are branched polymers containing dendritic branches having functional groups distributed on the periphery of the 30 branches, they can be prepared with a variety of properties. For example, starburst dendrimers, such as those depicted in Figure 2A and Figure 2B can have distinct properties due to the branch length. The dendrimer type shown in Figure 2A possesses 35 unsymmetrical (unequal segment) branch junctures, exterior (i.e., surface) groups (represented by Z'),

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interior moieties (represented by Z) but much less internal no internal void space. The dendrimer type shown in Figure 2B possesses symmetrical (equal segment) branch junctures with surface groups 5 (represented by Z'), two different interior moieties (represented respectively by X and Z) with interior void space which varies as a function of the generation (G). The dendrimers such as those depicted in Figure 2B can be advanced through enough generations to 10 totally enclose and contain void space, to give an entity with a predominantly hollow interior and a highly congested surface. Also, starburst dendrimers, when advanced through sufficient generations exhibit 15 "starburst dense packing" where the surface of the dendrimer contains sufficient terminal moieties such that the dendrimer surface becomes congested and encloses void spaces within the interior of the dendrimer. This congestion can provide a molecular 20 level barrier which can be used to control diffusion of materials into or out of the interior of the dendrimer.

Surface chemistry can be controlled in a predetermined fashion by selecting a repeating unit 25 which contains the desired chemical functionality or by chemically modifying all or a portion of the surface functionalities to create new surface functionalities. These surfaces may either be targeted toward specific sites or made to resist uptake by cells. In an 30 alternative use of the dendrimers, the dendrimers can themselves be linked together to create polydendric moieties (starburst "bridged dendrimers") which are also suitable as carriers.

35 In addition, the dendrimers can be prepared so as to have deviations from uniform branching in

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particular generations, thus providing a means of adding discontinuities (i.e., deviations from uniform branching at particular locations within the dendrimer) and different properties to the dendrimer.

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The starburst polymers employed in the starburst conjugates of the present invention can be prepared according to methods known in the art, for example, U. S. Patent 4,587,329.

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Dendrimers can be prepared having highly uniform size and shape and most importantly allow for a greater number of functional groups per unit of surface area of the dendrimer, and can have a greater number of 15 functional groups per unit of molecular volume as compared to other polymers which have the same molecular weight, same core and monomeric components and same number of core branches as the starburst polymers. The increased functional group density of 20 the starburst polymers may allow a greater quantity of material to be carried per dendrimer.

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An analogy can be made between early generation starburst dendrimers (i.e., generation = 1-7) to classical spherical micelles. The dendrimer - micelle analogy was derived by comparing features which they had in common such as shape, size and surface.

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TABLE I

	<u>Parameter</u>	<u>Regular Classical Micelles</u>	<u>Starburst Dendrimers</u>
5	Shape	Spherical	Spherical
	Size (diameter)	20-60Å	17-67Å
10	Surface		
	Aggregation Numbers	4-202	Z=6-192 (Generation = 2-7)
	Area/Surface Group (Å)	130-80Å ²	127-75Å ²

15 Z is the number of surface groups; 1Å = 10^{-1} nm;
 $1\text{Å}^2 = 10^{-2}$ nm².

20 In Table I, the shape was verified by scanning transmission electron micrographs (STEM) microscopy and intrinsic viscosity (η) measurements. The size was verified by intrinsic viscosity (η) and size exclusion chromatography (SEC) measurements. The surface aggregation numbers were verified by titremetry and high field NMR. The area/surface group was calculated from SEC hydrodynamic measurements.

30 The first five generations of starburst polyamidoamine (PAMAM) dendrimers are microdomains which very closely mimic classical spherical micelles in nearly every respect (i.e shape, size, number of surface groups, and area/surface group). A major difference, however, is that they are covalently fixed and robust compared to the dynamic equilibration of nature of micelles. This difference is a significant

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advantage when using these microdomains as controlled delivery prototypes or encapsulation devices.

As further concentric generations are added beyond five, congestion of the surface occurs. This congestion can lead to increased barrier characteristics at the surface and manifests itself as a smaller surface area per head (surface) group as shown in Table II.

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Table II
PAMAM Dendrimer Features vs. Generation

Generations	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>
# of surface groups, ²	3	6	12	24	48	96	192	384	768
Molecular wt.	275	875	2411	5147	10,619	21,563	43,541	87,227	174,779
Diameter*, measured SEC	10.4Å	15.8Å	22Å	31Å	40Å	53Å	67Å	76Å	88Å
Surface area per dendrimer	366Å ²	783Å ²	1519Å ²	3018Å ²	5024Å ²	8,820Å ²	14,096Å ²	18,136Å ²	36,083Å ²
Surface area per 2 group	122Å ²	131Å ²	127Å ²	126Å ²	104Å ²	92Å ²	73Å ²	47Å ²	32Å ²
Distance between 2 groups	12.4Å	12.8Å	12.7Å	12.6Å	11.5Å	10.8Å	9.8Å	7.75Å	6.28Å
Void Volume	311.6Å ³	1,470.2Å ³	4,737.9Å ³	11,427.0Å ³	---	---	---	---	---

* Hydrodynamic diameters determined by size exclusion chromatography measurements calibrated against monodisperse ($M_w = 1.02$) polyethyleneoxide standards.

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1Å = 10^{-1} nm; 1Å² = 10^{-2} nm²; 1 Å³ = 10^{-3} nm³.

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For example, amine terminated generations 5.0, 6.0, 7.0, 8.0 and 9.0 have decreased surface areas of 104, 92, 73, 47 and 32\AA^2 per Z group, respectively. This characteristic corresponds to a transition from a less congested micelle-like surface to a more congested bilayer/monolayer barrier-like surface normally associated with vesicles (liposomes) or Langmuir-Blodgett type membranes.

If this surface congestion is indeed occurring, the change in physical characteristics and morphology should be observed as the generations increase from the intermediate generations (6-8) to the more advanced generations (9 or 10). The scanning transmission electron micrographs (STEM) for generations = 7.0, 8.0 and 9.0 were obtained after removing the methanol solvent from each of the samples to provide colorless, light yellow solid films and staining with osmium-tetraoxide. The morphological change predicted occurred at the generation, G = 9.0 stage. The microdomains at generation = 9.0 measure about 33\AA in diameter and are surrounded by a colorless rim which is about 25\AA thick. Apparently, methanolic solvent has been entrapped within the 25\AA outer membrane-like barrier to provide the dark stained interior. Thus, at generation = 9.0, the starburst PAMAM is behaving topologically like a vesicle (liposome). However, this starburst is an order of magnitude smaller and very monodispersed compared to a liposome. Consequently, the present dendrimers can be used to molecularly encapsulate solvent filled void spaces of as much diameter as about 33\AA (volume about $18,000\text{\AA}^3$) or more. These micelle sized prototypes appear to behave like a covalently fixed liposome in this advanced generation

stage. These prototypes may have additional capability as carriers or as delivery agents.

Since the number of functional groups on the dendrimers can be controlled on the surface and within the interior, it also provides a means for controlling the amount of agricultural material to be delivered per dendrimer. In a particularly preferred embodiment of the present invention the dendrimers are targeted carriers of bioactive agents capable of delivering bioactive agent to a particular target organism such as a plant or pest or to a particular determinant or locus in a target organism. Dendrimers suitable for use in the conjugates of the present invention include the dense star polymers or starburst polymers described in U. S. Patents 4,507,466, 4,558,120, 4,568,737 and 4,587,329.

In particular, the present invention concerns a starburst conjugate which comprises at least one starburst polymer associated with at least one carried agricultural material. Starburst conjugates included within the scope of the present invention include those represented by the formula:



wherein each P represents a dendrimer;
x represents an integer of 1 or greater;
each M represents a unit (for example, a molecule, atom, ion, and/or other basic unit) of a carried agricultural material, said carried agricultural material can be the same carried agricultural material

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or a different carried agricultural material,
preferably the carried material is a bioactive agent;

y represents an integer of 1 or greater; and

5 * indicates that the carried material is associated
with the dendrimer.

Preferred starburst conjugates of formula (I)
are those in which M is a pesticide, radionuclide,
10 chelant, toxin, or signal generator, signal reflector,
or signal absorber; particularly preferred are those in
which x=1, and y=2 or more.

15 Also included are starburst conjugates of
formula (I) wherein the starburst dendrimers are
covalently linked together, optionally via linking
groups, so as to form polydendric assemblages (i.e.,
where x>1). Use of these starburst bridged dendrimers
20 include topical controlled release agents.

As used herein, "associated with" means that
the carried material(s) can be encapsulated or
entrapped within the core of the dendrimer, dispersed
25 partially or fully throughout the dendrimer, or
attached or linked to the dendrimer, or any combination
thereof. The association of the carried material(s)
and the dendrimer(s) may optionally employ connectors
and/or spacers to facilitate the preparation or use of
30 the starburst conjugates. Suitable connecting groups
represented by C', are groups which link a targeting
director (i.e., T) to the dendrimer (i.e., P) without
significantly impairing the effectiveness of the
director or the effectiveness of any other carried
35 material(s) (i.e., M) present in the starburst
conjugate. These connecting groups may be cleavable or

non-cleavable and are typically used in order to avoid steric hindrance between the target director and the dendrimer, preferably the connecting groups are stable (i.e., non-cleavable). Since the size, shape and 5 functional group density of the dense star dendrimers can be rigorously controlled, there are many ways in which the carried material can be associated with the dendrimer. For example, (a) there can be covalent, coulombic, hydrophobic, or chelation type association 10 between the carried material(s) and entities, typically functional groups, located at or near the surface of the dendrimer; (b) there can be covalent, coulombic, hydrophobic, or chelation type association between the 15 carried material(s) and moieties located within the interior of the dendrimer; (c) the dendrimer can be prepared to have an interior which is predominantly hollow allowing for physical entrapment of the carried materials within the interior (void volume); wherein 20 the release of the carried material can optionally be controlled by congesting the surface of the dendrimer with diffusion controlling moieties; or (d) various combinations of the aforementioned phenomena can be 25 employed.

Dendrimers, herein represented by "P", include the dense star polymers described in U. S. Patents 4,507,466; 4,558,120; 4,568,737 or 4,587,329.

30 Carried agricultural materials, including the term "agricultural materials", herein represented by "M", which are suitable for use in the starburst conjugates include any materials for in vivo or in vitro treatment, diagnosis, or application to plants 35 and non-mammals (including microorganisms) which can be associated with the starburst dendrimer without

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appreciably disturbing the physical integrity of the dendrimer. For example, carried materials like toxins such as diphtheria toxin, gelonin, exotoxin A, abrin, modeccin, ricin, or toxic fragments thereof; metal ions such as the alkali and alkaline-earth metals; radionuclides such as those generated from actinides or lanthanides or other similar transition elements or from other elements, such as ^{67}Cu , ^{90}Y , ^{111}In , ^{131}I , ^{186}Re , ^{105}Rh , $^{99\text{m}}\text{Tc}$, ^{67}Ga , ^{153}Sm , ^{159}Gd , ^{175}Yb , ^{177}Lu , ^{88}Y , ^{166}Ho , $^{115\text{m}}\text{In}$, ^{109}Pd , ^{82}Rb , ^{194}Ir , ^{140}Ba , ^{149}Pm , ^{199}Au , ^{140}La , and ^{188}Re ; signal generators such as fluorescing entities; signal reflectors such as paramagnetic entities; signal absorbers such as electron beam opacifiers; hormones; biological response modifiers such as interleukins, interferons, viruses and viral fragments; pesticides, including antimicrobials, algicides, arithelmetics, acaricides, insecticides, attractants, repellants, herbicides and/or fungicides such as acephate, acifluorfen, alachlor, atrazine, benomyl, bentazon, captan, carbofuran, chloropicrin, chloryprifos, chlorsulfuron cyanazine, cyhexatin, cypermetrin, 2,4-dichlorophenoxyacetic acid, dalapon, dicamba, diclofop methyl, diflubenzuron, dinoseb, endothall, ferbam, fluazifop, glyphosate, haloxyfop, malathion, naptalam, pendimethalin, permethrin, picloram, propachlor, propanil, sethoxydin, temephos, terbufos, trifluralin, triforine, zineb, and the like. Carried agricultural materials include scavenging agents such as chelants, chelated metal (whether or not they are radioactive), or any moieties capable of selectively scavenging therapeutic or diagnostic agents.

Preferably the carried materials are bioactive agents. As used herein, "bioactive" refers to an active entity such as a molecule, atom, ion and/or other entity which is capable of detecting, 5 identifying, inhibiting, treating, catalyzing, controlling, killing, enhancing or modifying a targeted entity such as a protein, glycoprotein, lipoprotein, lipid, a targeted cell, a targeted organ, a targeted organism [for example, a microorganism, plant, or animal 10 (excluding mammals)] or other targeted moiety.

The starbursts conjugates of formula (I) are prepared by reactive P with M, usually in a suitable solvent, at a temperature which facilitates the 15 association of the carried material (M) with the starburst dendrimer (P).

Suitable solvents are solvents in which P and M are at least partially miscible and inert to the 20 formation of the conjugate. If P and M are at least partially miscible with each other, no solvent may be required. When desired, mixtures of suitable solvents can be utilized. Examples of such suitable solvents 25 are water, methanol, ethanol, chloroform, acetonitrile, toluene, dimethylsulfoxide and dimethylformamide.

The reaction condition for the formation of the starburst conjugate of formula (I) depends upon the 30 particular dendrimer (P), the carried agricultural material (M), and the nature of the bond (*) formed. For example if P is the PEI (polyethyleneimine) starburst dendrimer with a methylene carboxylate surface, M is a radionuclide, e.g. yttrium, then the 35 reaction is conducted at room temperature in water. Typically, the temperature can range from room

temperature to reflux. The selection of the particular solvent and temperature will be apparent to one skilled in the art.

5 The ratio of M:P will depend on the size of the dendrimer and the amount of carried material. For example, the molar ratio (ratio of moles) of any ionic M to P is usually 0.1-1,000:1, preferably 1-50:1, and more preferably 2-6:1. The weight ratio of any
10 pesticide or toxin M to P is usually 0.1-5:1, and preferably 0.5-3:1.

When M is a radionuclide, there are three ways the starburst conjugate can be prepared, namely: (1) P
15 can be used as a chelant. For example a methylenecarboxylate surface PEI or PAMAM will chelate a metal such as yttrium or indium. (2) A chelate can be covalently bonded to P. For example, an amine terminated PEI starburst dendrimer can be reacted with
20 1-(p-isothiocyanatobenzyl)diethylenetriaminepenta-acetic acid and then chelated, or a complex such as rhodium chloride chelated with isothiocyanatobenzyl-
2,3,2-tet can be reacted. (3) A prechelated
25 radionuclide can be associated with P by hydrophobic or ionic interaction.

Other starburst conjugates are those conjugates which contain a target director (herein designated as
30 "T") and which are represented by the formula:

$$(T)_e * (P)_x * (M)_y \quad (\text{II})$$

35 wherein

each T represents a target director;

e represents an integer of 1 or greater; and

P, x, *, M, and y are as previously defined herein.

5

Preferred among the starburst conjugates of formula (II) are those in which M is a pesticide, radionuclide, chelator, chelated metal, toxin, signal generator, signal reflector, or signal absorber. Also preferred 10 are those conjugates in which e=1 or 2; and those in which x=1 and y=2 or more. Particularly preferred conjugates are those in which x=1, e=2, y=2 or more and M and T are associated with the polymer via the same or 15 different connectors.

15

The starburst conjugates of formula (II) are prepared either by forming T^*P and then adding M or by forming P^*M and then adding T. Either reaction scheme 20 is conducted at temperatures which are not detrimental to the particular conjugate component and in the presence of a suitable solvent when required. To control pH, buffers or addition of suitable acid base 25 is used. The reaction conditions are dependent on the type of association formed (*), the starburst dendrimer used (P), the carried agricultural material (M), and the target director (T). Alternatively, P and M can be chelated, usually in water, before conjugation to T. The conjugation with T is carried out in a suitable 30 buffer.

The ratio of T:P is preferably 1:1. The ratio of M:P will be as before.

35

Target directors capable of targeting the starburst conjugates are entities which when used in

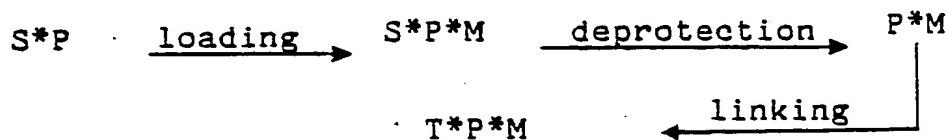
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the starburst conjugates of the present invention result in at least a portion of the starburst conjugates being delivered to a desired target (for example, a protein, glycoprotein, lipoprotein, lipid, a 5 targeted cell, a targeted organism or other targeted moiety) and include hormones, biological response modifiers, chemical functionalities exhibiting target specificity, and the like.

10 In the absence of a target director (or in the presence of a target director if desired), due to the number of functional groups which can be located at or near the surface of the dendrimer, all or a substantial portion of such functional groups can be made anionic, 15 cationic, hydrophobic or hydrophilic to effectively aid delivery of the starburst conjugate to a desired target of the opposite charge or to a hydrophobic or hydrophilic compatible target.

20 Preparation of the conjugates of formula (II) using a P with a protected handle (S) is also intended as a process to prepare the conjugates of formula (II). The reaction scheme is shown below:

25



30

where

S*P represents the protected dendrimer;

35 S*P*M represents the protected dendrimer conjugated with m;

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P*M represents the dendrimer conjugated with M (starburst conjugate);

T*P*M represents the starburst conjugates linked to the target director.

5

Suitable solvents can be employed which do not effect P*M. For example when S is t-butoxycarbamate, S can be removed by aqueous acid.

10

The starburst conjugates can be used for a variety of in vivo and in vitro diagnostic applications pertaining to plants and non-mammals, such as radioimmunoassays, electron microscopy, enzyme linked immunosorbent assays, nuclear magnetic resonance

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spectroscopy, contrast imaging, and immunoscintigraphy, in analytical applications; and in biological control applications as a means of delivering pesticides such as herbicides, insecticides, fungicides, repellants, attractants, repellants, attractants, antimicrobials or other toxins, or used as starting materials for making other useful agents.

20

The present invention is also directed to starburst conjugate compositions in which the starburst conjugates are formulated with other suitable vehicles useful in agriculture such as on crops, fallow land, or as pesticides, or in treatment of or in vivo or in vitro testing of non-mammals. The starburst conjugate 30 compositions may optionally contain such other active ingredients, additives and/or diluents.

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An agriculturally acceptable carrier or diluent which may also be present with one or more starburst conjugates of the present invention includes those carriers or diluents customarily used in granular

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formulations, emulsifiable concentrates, solutions, or suspensions such as, for example, toluene, xylene, benzene, phenol, water, methane, hydrocarbons, naphthalene and others.

5 The preferred starburst polymer for use in the starburst conjugates of the present invention is a polymer that can be described as a starburst having at least one branch (hereinafter called a core branch),
10 preferably two or more branches, emanating from a core, said branch having at least one terminal group provided that (1) the ratio of terminal groups to the core branches is more than one, preferably two or greater,
15 (2) the density of terminal groups per unit volume in the polymer is at least 1.5 times that of an extended conventional star polymer having similar core and monomeric moieties and a comparable molecular weight and number of core branches, each of such branches of
20 the extended conventional star polymer bearing only one terminal group, and (3) a molecular volume that is no more than about 80 percent of the molecular volume of said extended conventional star polymer as determined by dimensional studies using scaled Corey-Pauling
25 molecular models. As used herein, the term "dense" as it modifies "star polymer" or "dendrimer" means that it has a smaller molecular volume than an extended conventional star polymer having the same molecular weight. The extended conventional star polymer which
30 is used as the base for comparison with the starburst polymer is one that has the same molecular weight, same core and monomeric components and same number of core branches as the starburst polymer. By "extended" it is
35 meant that the individual branches of the conventional star polymer are extended or stretched to their maximum

length, e.g., as such branches exist when the star polymer is completely solvated in an ideal solvent for the star polymer. In addition while the number of terminal groups is greater for the starburst polymer molecule than in the conventional star polymer molecule, the chemical structure of the terminal groups is the same.

Dendrimers used in the conjugates of the present invention can be prepared by processes known in the art. The above dendrimers, the various coreactants and core compounds, and process for their preparation can be as defined in U. S. Patent 4,587,329.

The starburst dendrimers, for use in the starburst conjugates of the present invention, can have terminal groups which are sufficiently reactive to undergo addition or substitution reactions. Examples of such terminal groups include amino, hydroxy, mercapto, carboxy, alkenyl, allyl, vinyl, amido, halo, urea, oxiranyl, aziridinyl, oxazolinyl, imidazolinyl, sulfonato, phosphonato, isocyanato and isothiocyanato. The terminal groups can be modified to make them biologically inert. The dendrimers differ from conventional star or star-branched polymers in that the dendrimers have a greater concentration of terminal groups per unit of molecular volume than do conventional extended star polymers having an equivalent number of core branches and an equivalent core branch length. Thus, the density of terminal groups per unit volume in the dendrimer usually is at least about 1.5 times the density of terminal groups in the conventional extended star polymer, preferably at least 5 times, more preferably at least 10 times, most preferably from 15 to 50 times. The ratio of terminal

-22-

groups per core branch in the starburst dendrimer is preferably at least 2, more preferably at least 3, most preferably from 4 to 1024. Preferably, for a given polymer molecular weight, the molecular volume of the
5 starburst dendrimer is less than 70 volume percent, more preferably from 16 to 60, most preferably from 7 to 50 volume percent of the molecular volume of the conventional extended star polymer.

10 Preferred starburst dendrimers for use in the starburst conjugates of the present invention are characterized as having a univalent or polyvalent core that is covalently bonded to dendritic branches. Such ordered branching can be illustrated by the following
15 sequence wherein G indicates the number of generations:

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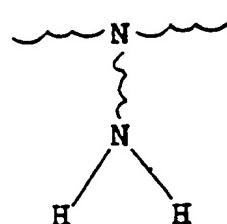
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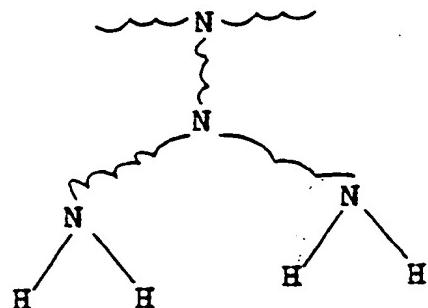
-23-

 $G = 1$

5



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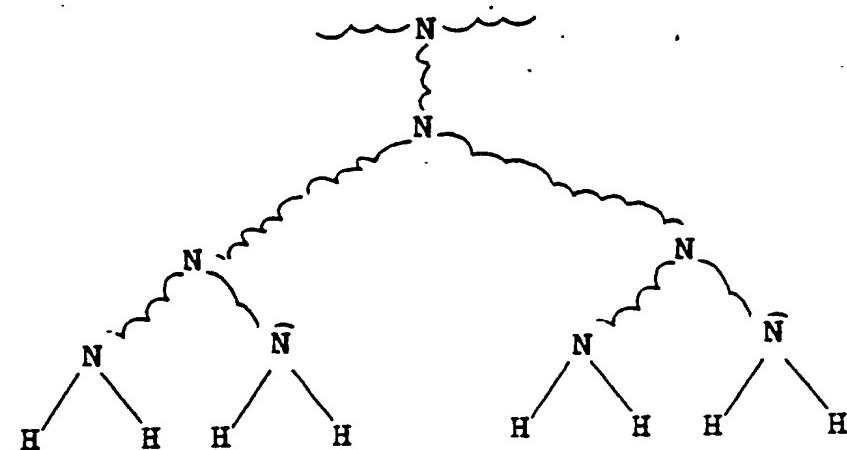
 $G = 2$ 

15

 $G = 3$

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-24-

Mathematically, the relationship between the number (#) of terminal groups on a dendritic branch and the number of generations of the branch can be represented as follows:

5

$$\# \text{ of terminal groups per dendritic branch} = \frac{N_r^G}{2}$$

10

wherein G is the number of generations and N_r is the repeating unit multiplicity which is at least 2 as in the case of amines. The total number of terminal groups in the dendrimer is determined by the following:

15

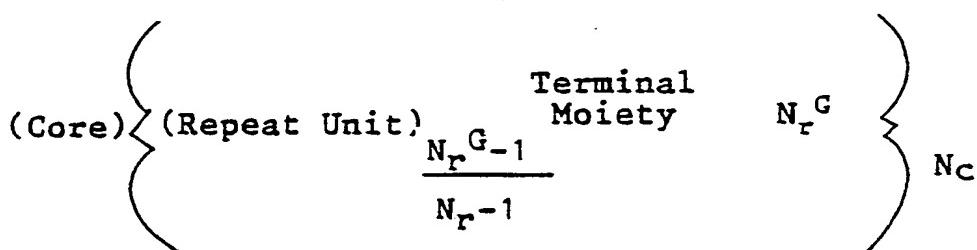
$$\# \text{ of terminal groups per dendrimer} = \frac{N_c N_r^G}{2}$$

20

wherein G and N_r are as defined before and N_c represents the valency (often called core functionality) of the core compound. Accordingly, the dendrimers of this invention can be represented in its component parts as follows:

25

30



35

wherein the Core, Terminal Moiety, G and N_c are as defined before and the Repeat Unit has a valency or

functionality of $N_r + 1$ wherein N_r is as defined before.

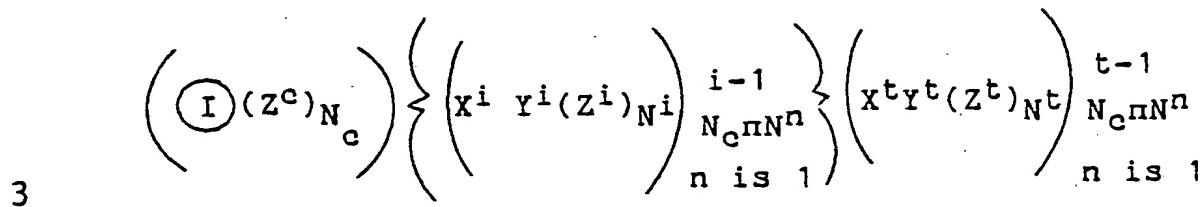
A copolymeric dendrimer which is a preferred dendrimer for the purposes of this invention is a unique compound constructed of polyfunctional monomer units in a highly branched (dendritic) array. The dendrimer molecule is prepared from a polyfunctional initiator unit (core compound), polyfunctional repeating units and terminal units which may be the same or different from the repeating units. The core compound is represented by the formula $I(Z^c)_{N_c}$ wherein I represents the core, Z^c represents the functional groups bonded to I and N_c represents the core functionality which is preferably 2 or more, most preferably 3 or more. Thus, the dendrimer molecule comprises a polyfunctional core, I , bonded to a number (N_c) of functional groups, Z^c , each of which is connected to the monofunctional tail of a repeating unit, $x^1y^1(z^1)_{N^1}$, of the first generation and each of the Z groups of the repeating unit of one generation is bonded to a monofunctional tail of a repeating unit of the next generation until the terminal generation is reached.

In the dendrimer molecule, the repeating units are the same within a single generation, but may differ from generation to generation. In the repeating unit, $x^1y^1(z^1)_{N^1}$, x^1 represents the monofunctional tail of the first generation repeating unit, y^1 represents the moiety constituting the first generation, z^1 represents the functional group of the polyfunctional head of the repeating unit of the first generation and may be the same as or different from the functional groups of the core compound, $I(Z^c)_{N_c}$, or other generations; and N^1

-26-

is a number of 2 or more, most preferably 2, 3 or 4, which represents the multiplicity of the polyfunctional head of the repeating unit in the first generation. Generically, the repeating unit is represented by the 5 formula $X^i Y^i (Z^i)_{N^i}$ wherein "i" represents the particular generation from the first to the t-1 generation. Thus, in the preferred dendrimer molecule, each Z^1 of the first generation repeating unit is connected to an X^2 of a repeating unit of the second 10 generation and so on through the generations such that each Z^i group for a repeating unit $X^i Y^i (Z^i)_{N^i}$ in generation number "i" is connected to the tail (X^{i+1}) of the repeating unit of the generation number "i+1". 15 The final or terminal of a preferred dendrimer molecule comprises terminal units, $X^t Y^t (Z^t)_{N^t}$ wherein t represents terminal generation and X^t , Y^t , Z^t and N^t may be the same as or different from X^i , Y^i , Z^i and N^i except that there is no succeeding generation connected 20 to the Z^t groups and N^t may be less than two, e.g., zero or one. Therefore the preferred dendrimer has a molecular formula represented by

22



where i is 1 to t-1

-27-

wherein the symbols are as previously defined. The π function is the product of all the values between its defined limits. Thus

5 $\prod_{n=1}^{i-1} N^n = (N^1)(N^2)(N^3)\dots(N^{i-2})(N^{i-1})$

which is the number of repeat units, $x_i y_i (Z^i)_{N^i}$, comprising the i th generation of one dendritic branch
10 and when i is 1, then

15 $\prod_{n=1}^0 = 1$

In copolymeric dendrimers, the repeat unit for one generation differs from the repeat unit in at least one other generation. The preferred dendrimers are very symmetrical as illustrated in structural formulas described hereinafter. Preferred dendrimers may be converted to functionalized dendrimers by contact with another reagent. For example, conversion of hydroxyl in the terminal generation to ester by reaction with an acid chloride gives an ester terminally functionalized dendrimer. This functionalization need not be carried out to the theoretical maximum as defined by the number of available functional groups and, thus, a functionalized dendrimer may not have high symmetry or a precisely defined molecular formula as is the case with the preferred dendrimer.

35 In a homopolymer dendrimer, all of the repeat units, $x_i y_i (Z^i)_{N^i}$, are identical. Since the values of

-28-

all N^i are equal (defined as N_r), the product function representing the number of repeat units reduces to a simple exponential form. Therefore, the molecular formula may be expressed in simpler form as

5

$$10 \left(\bigcirc_I (Z^c)_{N_c} \right) \left\{ \left(x^i y^i (Z^i)_{N^i} \right)_{N_c N_r^{i-1}} \right\} \left(x^t y^t (Z^t)_{N^t} \right)_{N_c N_r^{t-1}}$$

where $i = 1$ to $t-1$

15

This form still shows the distinction between the different generations i , which each consist of $N_c N_r^{(i-1)}$ repeating units, $x^i y^i (Z^i)_{N^i}$. Combining the 20 generations into one term gives:

25

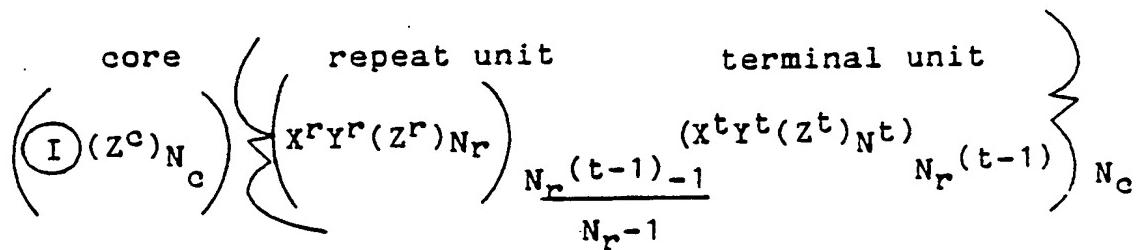
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$$\left(\textcircled{I} (Z^c)_{N_c} \right) \left(X^r Y^r (Z^r)_{N_r} \right)_{N_c} \underbrace{N_r}_{N_r-1}^{(t-1)-1} \left(X^t Y^t (Z^t)_{N_t} \right)_{N_c N_r^{t-1}}$$

or



wherein $X^r Y^r (Z^r)_{N_r}$ is the repeating unit which is used in all generations i.

20

Consequently, if a polymer compound will fit into these above formulae, then the polymer is a starburst polymer. Conversely, if a polymer compound will not fit into these above formulae, then the 25 polymer is not a starburst polymer. Also, to determine whether a polymer is a starburst polymer, it is not necessary to know the process by which it was prepared, but only whether it fits the formulae. The formulae 30 also demonstrate the generations (G) or tiering of dendrimers.

25

Clearly, there are several ways to determine the ratio of agent (M) to dendrimer (P) which depend upon how and where the association of P^*M occurs. When 35 there is interior encapsulation, the weight ratio of

-30-

M:P usually is 10:1, preferably 8:1, more preferably 5:1, most preferably 3:1. The ratio can be as low as 0.5:1 to 0.1:1. When interior stoichiometry is used, the weight ratio of M:P is the same as for interior
5 encapsulation. When exterior stoichiometry is determined, the mole/mole ratio of M:P given by the following formulae:

10

M

:

P

15

(A)	$5 N_c N_t N_r G^{-1}$	1
(B)	$3 N_c N_t N_r G^{-1}$	1
(C)	$1 N_c N_t N_r G^{-1}$	1

20

25

where N_c means the core multiplicity, N_t means the terminal group multiplicity, and N_r means branch juncture multiplicity. The $N_c N_t N_r G^{-1}$ term will result in the number of Z groups. Thus, for example, (A) above may result when proteins, enzymes or highly charged molecules are on the surface; (B) above when it is 2,4-D or octanoic acid; (C) above when converting surface ester groups to carboxylate ion's or groups.

30

35

Of course other structures of various dimensions can be readily prepared by one skilled in the art by appropriately varying the dendrimer components and number of generations employed. The dimensions of the dendrimers are significant in that they are small. A linear polymer of comparable molecular weight would have a radius of gyration, (in its fully extended form), that would be much larger than the same molecular weight dendrimer.

Linking target directors to dendrimers is another aspect of the present invention. In preferred embodiments of the present invention, a reactive functional group such as a carboxyl, sulfhydryl, 5 reactive aldehyde, reactive olefinic derivative, isothiocyanato, isocyanato, amino, reactive aryl halide, or reactive alkyl halide can conveniently be employed on the dendrimer. The reactive functional groups can be introduced to the dendrimer using known 10 techniques, for example:

(1) Use of a heterofunctional initiator (as a starting material for synthesizing the dendrimer) which has incorporated into it functional groups of different 15 reactivity. In such heterofunctional initiator at least one of the functional groups will serve as an initiation site for dendrimer formation and at least one of the other functional groups will be available 20 for linking to a target director but unable to initiate dendrimer synthesis. For example, use of protected aniline to allow further modification of NH₂ groups within the molecule without reacting the aniline NH₂.

The functional group which will be available 25 for linking to a target director may be part of the initiator molecule in any one of three forms namely:

- (a) In the form in which it will be used for 30 linking with the target director. This is possible when none of the synthetic steps involved in the dendrimer synthesis can result in reaction at this center.
- (b) When the functional group used for linking 35 to the targeting director is reactive in

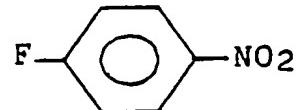
the synthetic steps involved in the dendrimer synthesis, it can be protected by use of a protecting group, which renders the group unreactive to the synthetic procedures involved, but can itself be readily removed in a manner which does not alter the integrity of the remainder of the macromolecule.

- 5
- 10 (c) In the event that no simple protecting group can be found for the reactive functionality to be used for linking with the targeting director, a synthetic precursor can be used which is unreactive in all the synthetic procedures used in the dendrimer synthesis. On completion of the synthesis, this functional group must be readily convertible into the desired linking group in a manner which does not alter the integrity of the remainder of the molecule.
- 15
- 20
- 25 (d) Coupling (covalently) the desired reactive functional group onto a preformed dendrimer. The reagent used must contain a functionality which is readily reacted with the terminal functional groups of the dendrimer. The functional group to be ultimately used to link with the targeting agent can be in its final form, as a protected functionality, or as a synthetic precursor. The form in which this linking functionality is used depends on its integrity during the synthetic procedure to be utilized, and the ability of the
- 30
- 35

-33-

final macromolecule to withstand any conditions necessary to make this group available for linking.

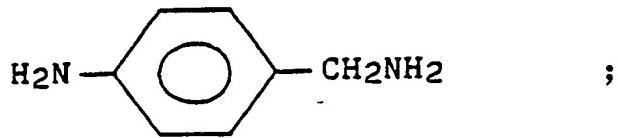
5 For example, the preferred route for PEI uses



10

Examples of heterofunctional initiators for use in (1) above, include the following illustrative examples:

15



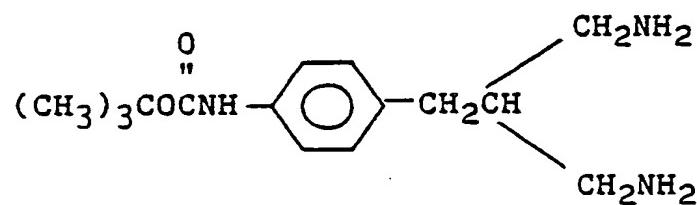
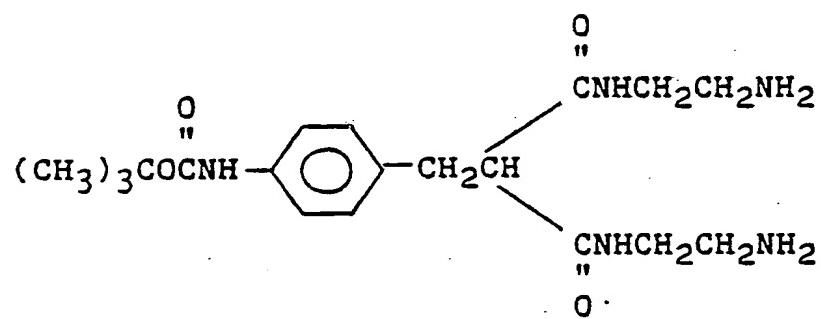
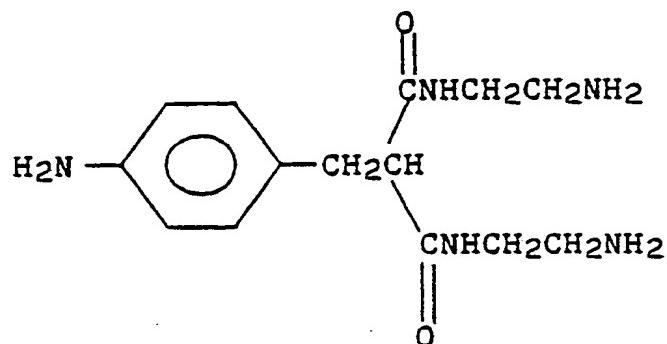
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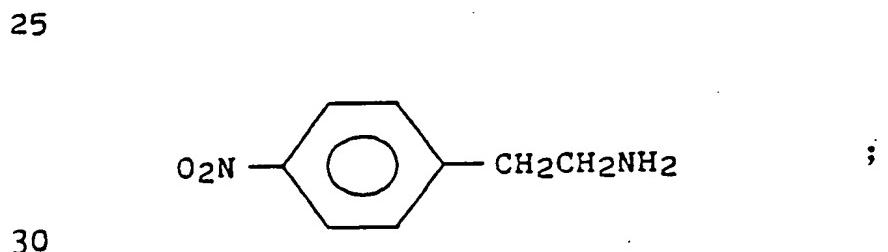
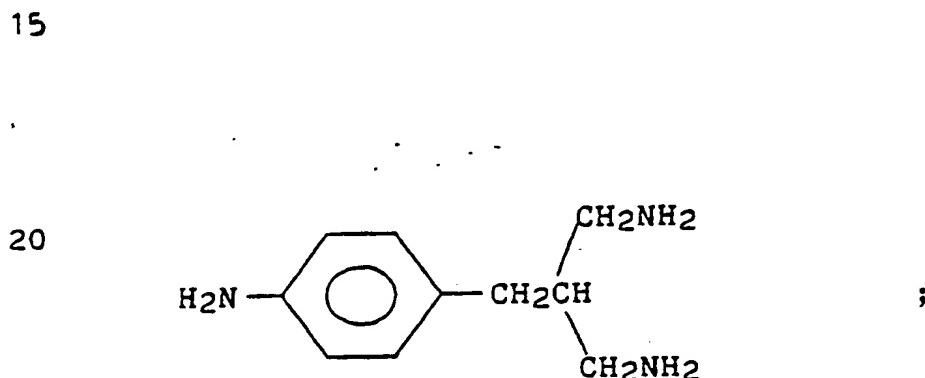
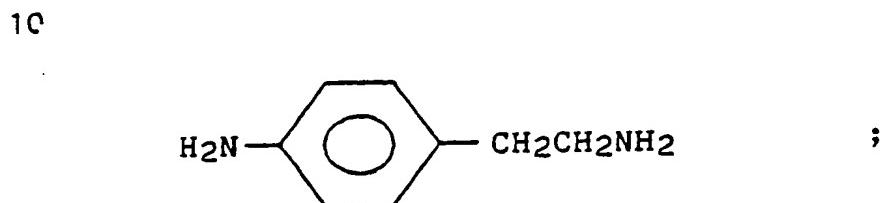
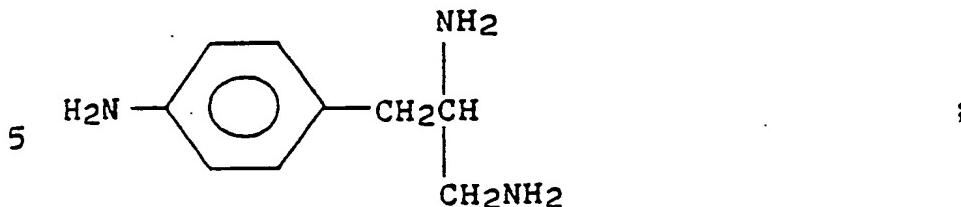
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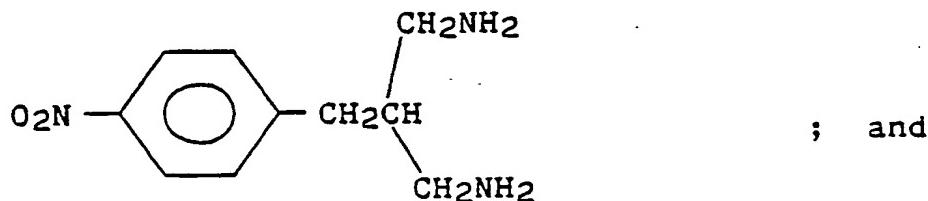
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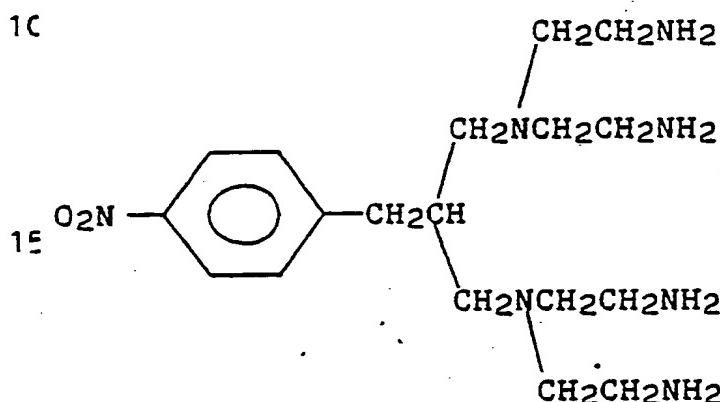
-36-

5



; and

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15

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25

There are several chemistries of particular importance:

30

- 1) Starburst Polyamidoamides ("PAMAM") Chemistry;
- 2) Starburst Polyethyleneimines ("PEI") Chemistry;
- 3) Starburst PEI compound with a surface of PAMAM;
- 4) Starburst polyether ("PE") chemistry.

Modifications of the dendrimer surface functionalities may provide other useful functional

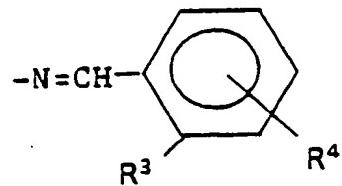
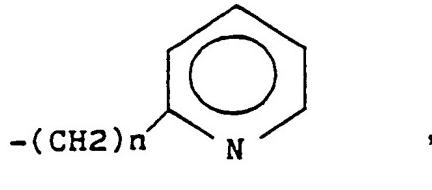
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groups such as the following:

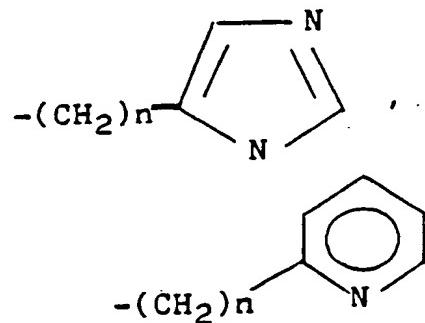
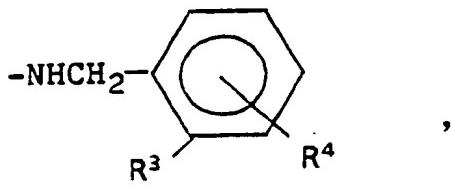
-OP₀₃H₂, -PO₃H₂, -PO₃H⁽⁻¹⁾, -PO₃⁽⁻²⁾, -CO₂⁽⁻¹⁾, -SO₂H,
-SO₂⁽⁻¹⁾, -SO₃H, -SO₃⁽⁻¹⁾, -NR¹R², -R⁵, -OH, -OR¹,
5 -NH₂, polyethers, perfluorinated alkyl, -CNHR¹, -COH,
" " O O

10



15

20



25

wherein

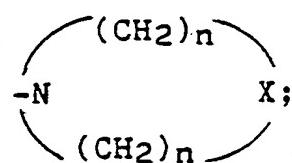
R represents alkyl, aryl or hydrogen;

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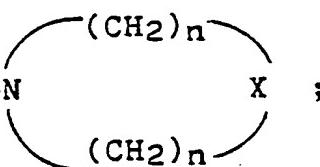
-38-

R¹ represents alkyl, aryl, hydrogen, or



5

R² represents alkyl, aryl, or -N



10 R³ represents -OH, -SH, -CO₂H, -SO₂H, or -SO₃H;

10 R⁴ represents alkyl, aryl, alkoxy, hydroxyl, mercapto, carboxyl, nitro, hydrogen, bromo, chloro, iodo, or fluoro;

15 R⁵ represents alkyl;

x represents NR, O or S; and

n represents the integer 1, 2 or 3.

20 The choice of functional group depends upon the particular end use for which the dendrimer is designed.

25 The following examples further illustrate the invention but are not to be construed as a limitation on the scope of the invention. The lettered examples concern the preparation of starting materials; the numbered examples concern the preparation of products.

30 Example A: Preparation of 2-Carboxamido-3-(4'-nitro-phenyl)-propanamide.

35 p-Nitrobenzyl malonate diethylester (2.4 grams (g), 8.13 mmole) was dissolved in 35 ml of methanol. The solution was heated to 50-55°C with stirring and a stream of anhydrous ammonia was bubbled through the solution for 64 hours. The solution was cooled and the

white, flocculant product was filtered and recrystallized from 225 milliliters (ml) of boiling methanol to afford 1.85 g (7.80 mmole) of bis amide in 96% yield (mp = 235.6°C(d)).

5

The structure was confirmed by MS, ¹H and ¹³C NMR spectroscopy.

Anal: Calc. for C₁₀H₁₁O₄N₃

10

	<u>C</u>	<u>H</u>	<u>N</u>
Theo:	50.63	4.69	17.72
Found:	50.75	4.81	17.94

15

Example B: Preparation of 1-Amino-2-(aminomethyl)-3-(4'-nitrophenyl)propane.

20

2-Carboxamido-3-(4' nitrophenyl)propanamide (2.0 g, 8.43 mmole) was slurried in 35 ml of dry tetrahydrofuran under a nitrogen atmosphere with stirring. To this mixture was added borane/tetrahydrofuran complex (106 ml, 106 mmole) via syringe. The reaction mixture was then heated to reflux for 48 hours during which time the suspended amide dissolved. The solution was cooled and the tetrahydrofuran was removed in vacuo using a rotary evaporator. The crude product and borane residue was dissolved in 50 ml of ethanol and this solution was purged with anhydrous hydrogen chloride gas. The solution was refluxed for 1 hour and the solvent removed in vacuo. The crude hydrochloride salt was dissolved in 15 ml of deionized water and extracted with two 50 ml portions of methylene chloride. The aqueous layer was cooled in an ice bath under an argon blanket and 50% sodium hydroxide was slowly added until basic pH=11.7. The basic aqueous layer was extracted with four 25 ml portions of

-40-

methylene chloride and these combined extracts were evaporated (rotary) to give 1.45 g of amber colored oil. This oil was triturated with diethyl ether (50 ml) and filtered under pressure through a short silica gel (grade 62 Aldrich) column. The column was washed with 100 ml of ether and the combined filtrates were vacuum evaporated giving 1.05 g (5.02 mmole) of the titled diamine as a clear oil (mp = 275-278°C(d) bis HCl salt).

10

The structure was confirmed by MS, ¹H and ¹³C NMR spectroscopy.

Anal: Calc. for C₁₀H₁₇N₃O₂Cl₂

15

	C	H	N
Theo:	42.57	6.07	14.89
Found:	43.00	6.14	15.31

20

Example C: Preparation of 1-Amino-2-(aminomethyl)-3-(4'-aminophenyl)propane.

25

30

35

Borane/tetrahydrofuran solution (70 ml, 70 mmole) was added under nitrogen via a cannula needle to a flask containing 4-amino-benzyl malonamide (1.5 g, 7.24 mmole) with stirring. The solution was brought to reflux for 40 hours. The colorless solution was cooled and excess tetrahydrofuran was removed by rotary evaporation leaving a clear gelatinous oil. Methanol (50 ml) was cautiously added to the oil with notable gas evolution. Dry hydrogen chloride was bubbled through the suspension to effect dissolution and the solution was then refluxed for 1 minute. The methanol/HCl was rotary evaporated and the resulting hydrochloride salt was carried through the same dissolution/reflux procedure again. The hydrochloride

-41-

salt obtained was dissolved in 10 ml of water and cooled in an ice bath under argon. Concentrated sodium hydroxide (50%) was added slowly with stirring to pH=11. The aqueous portion was then extracted with 2 X 5 100 ml portions of chloroform which were combined and filtered through a short silica gel plug without drying. The solvent was removed in vacuo (rotary) affording the title compound (0.90 g, 5.02 mmole) in 70% yield ($R_f = 0.65$ - $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ conc - 2/2/1).
10 The structure was confirmed by ^1H and ^{13}C NMR and used without further purification.

Example D: Preparation of 6-(4-Aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane.

15 4-Aminobenzyl malonate dimethylester (2.03 g, 8.43 mmole) was dissolved in 10 ml of methanol. This solution was added dropwise to a stirred solution of freshly distilled ethylene diamine (6.00 g, 103.4 mmole) in 20 10 ml of methanol under nitrogen over a 2 hour period. The clear solution was stirred for 4 days and Thin Layer Chromotography (TLC) analysis indicated total conversion of diester ($R_f = 0.91$) to the bis amide ($R_f = 0.42$ - 20% conc $\text{NH}_4\text{OH}/80\%$ ethanol). This 25 material was strongly ninhydrin positive. The methanol and excess diamine were removed on a rotary evaporator and the resulting white solid was vacuum dried (10^{-1} mm, 50°C) overnight to afford crude product (2.45g, 8.36 mmole) in 99% yield. An analytical sample was recrystallized from chloroform/hexane, MP = 160-161°C. The mass spectral, ^1H and ^{13}C NMR data were consistent 30 with the proposed structure.

-42-

Example E: Reaction of Mesyl Aziridine with 1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)propane.

1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)-
5 propane (400 mg, 1.91 mmole, >96% pure) was dissolved
in 10.5 ml of absolute ethanol under nitrogen. Mesyl
aziridine (950 mg, 7.85 mmole) was added to the stirred
diamine solution as a solid. The reaction was stirred
at 25°C for 14 hours using a magnetic stirrer and during
this period a white, gummy residue formed on the sides
10 of the flask. The ethanol was decanted and the residue
was triturated with another 15 ml portion of ethanol to
remove any unreacted aziridine. The gummy product was
vacuum dried (10¹mm, 25°C) to afford the tetrakis methyl
15 sulfonamide (1.0 g, 1.44 mmole) in 75% yield ($R_f = 0.74$
- NH₄OH/ethanol - 20/80). The structure was confirmed
by ¹H and ¹³C nuclear magnetic resonance (NMR)
spectroscopy.

20 Example F: Preparation of 2-(4-Nitrobenzyl)-1,3-(bis-
N,N-2-aminoethyl)diaminopropane.

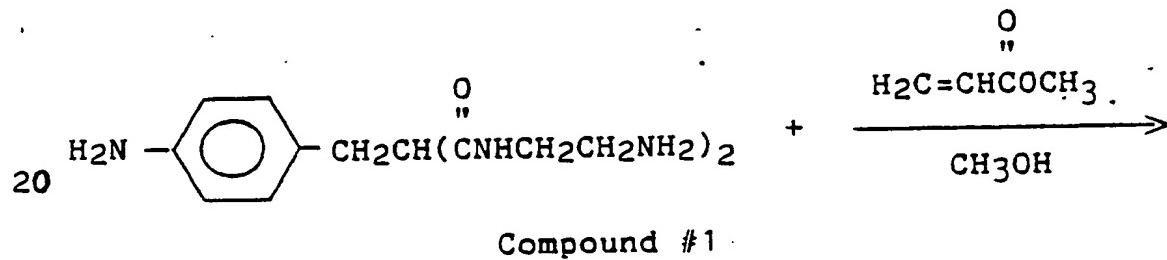
The crude methylsulfonamide (650 mg, 0.94
mmole) was dissolved in 5 ml of nitrogen purged,
25 concentrated sulfuric acid (98%). This solution was
maintained under nitrogen and heated to 143-146°C for 27
minutes with vigorous stirring. A slight darkening was
noted and the cooled solution was poured into a stirred
solution of ether (60 ml). The precipitated white salt
30 cake was filtered and immediately dissolved in 10 ml of
deionized water. The pH of the solution was adjusted
to pH=11 with 50% NaOH under argon with cooling. The
resulting solution was mixed with 90 ml of ethanol and
the precipitated inorganic salts were filtered. The
35 solvent was removed from the crude amine under reduced
pressure and to the resulting light brown oil was added

-43-

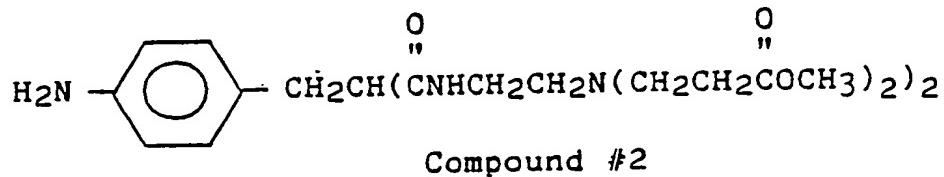
190 ml of toluene under nitrogen. The mixture was stirred vigorously and water was removed through azeotropic distillation (Dean-Stark trap) until the remaining toluene acquired a light yellow color (30-40 ml remaining in pot). The toluene was cooled and decanted from the dark, intractable residues and salt. This solution was stripped of solvent in vacuo and the resulting light yellow oil was vacuum dried (0.2 mm, 35°C) overnight affording 210 mg of the product (60%) which was characterized by MS, ¹H and ¹³C NMR.

Example G: Preparation of a starburst polymer (containing an aniline derivative) of one half generation represented by the following scheme:

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Methyl acrylate (2.09 g, 24 mmole) was dissolved in methanol (15 ml). The compound 6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (1.1 g, 3.8 mmole) (i.e., Compound #1) was dissolved in methanol (10 ml) and was added slowly over 2 hours with rigorous stirring to the methyl acrylate solution. The

35

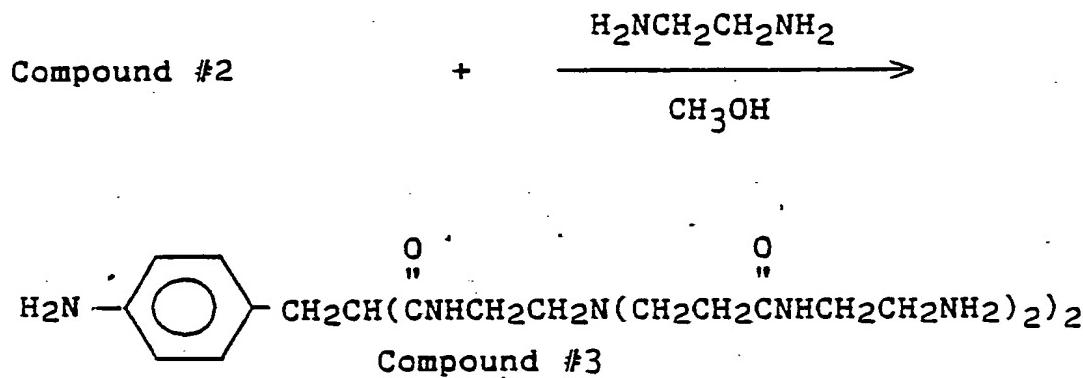
-44-

reaction mixture was stirred for 48 hours at ambient temperatures. The solvent was removed on the rotary evaporator maintaining the temperature below 40°C. The ester (Compound #2) was obtained as a yellow oil (2.6 g). No carboxyethylation of the aniline function was observed.

Example H: Preparation of a starburst polymer (containing an aniline moiety) of one generation; represented by the following scheme:

15

2

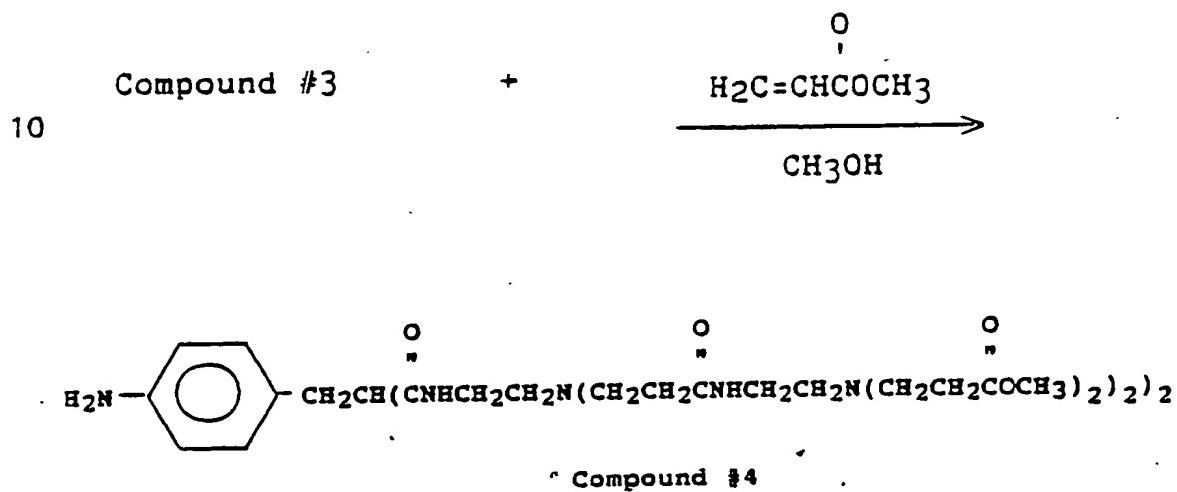


The ester (Compound #2) (2.6 g, 3.7 mmole) was dissolved in methanol (100 ml). This was carefully added to a vigorously stirring solution of ethylene diamine (250 g, 4.18 mole) and methanol (100 ml) at such a rate that the temperature did not rise above 40°C. After complete addition the reaction mixture was stirred for 28 hours at 35-40°C (heating mantle). After 25 28 hours no ester groups were detectable by infrared spectroscopy. The solvent was removed on the rotary evaporator at 60°C. The excess ethylene diamine was removed using a ternary azeotrope of toluene-methanol-ethylene diamine. Finally all remaining toluene was 30 35 azeotroped with methanol. Removal of all the methanol

-45-

yielded 3.01 g of the product (Compound #3) as an orange glassy solid.

Example I: Preparation of a starburst polymer (containing an aniline moiety) of one and one half generations represented by the following scheme:

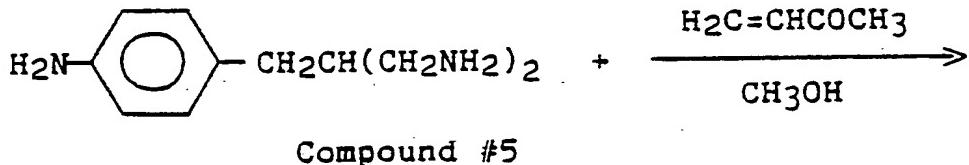


20 The amine (Compound #3) (2.7 g, 3.6 mmole) was dissolved in methanol (7 ml) and was added slowly over one hour to a stirred solution of methyl acrylate (3.8 g, 44 mmole) in methanol (15 ml) at ambient temperatures. A slight warming of the solution was observed during the addition. The solution was allowed to stir at ambient temperatures for 16 hours. The solvent was removed on the rotary evaporator at 40°C. After removal of all the solvent and excess methyl acrylate the ester (Compound #4) was obtained in: 4.7 g yield as an orange oil.

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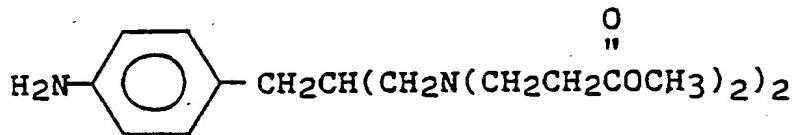
Example J: Preparation of a starburst polymer
 (containing an aniline moiety) of one half generation
 represented by the following scheme:

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Compound #6

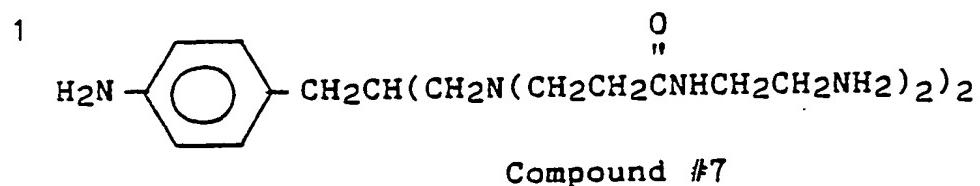
The triamine (Compound #5, the preparation of this compound is shown in Example C) (0.42 g, 2.3 mmole) was dissolved in methanol (10 ml) and was added dropwise over one hour to methyl acrylate (1.98 g, 23 mmole) in methanol (10 ml). The mixture was allowed to stir at ambient temperatures for 48 hours. The solvent was removed on the rotary evaporator, maintaining the temperature at no higher than 40°C. The excess methyl acrylate was removed by repeated azeotroping with methanol. The ester (Compound #6) was isolated as an orange oil (1.24 g).

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Example K: Preparation of a starburst polymer (containing an aniline moiety) of one generation; represented by the following scheme:



1 The ester (Compound #6) (1.24 g, 2.3 mmole) was
 dissolved in methanol (50 ml) and was added dropwise
 over two hours to ethylenediamine (73.4 g, 1.22 mole)
 in methanol (100 ml). A small exotherm was noted,
 20 vigorous stirring was maintained. The solution was
 left to stir at ambient temperatures for 72 hours. The
 solvent was removed on the rotary evaporator at 60°C.
 25 The excess ethylene diamine was removed using a ternary
 azeotrope of toluene-methanol-ethylenediamine. Finally
 all remaining toluene was removed with methanol and
 then pumping down with a vacuum pump for 48 hours gave
 the amine (Compound #7) (1.86 g) as a yellow/orange
 oil.

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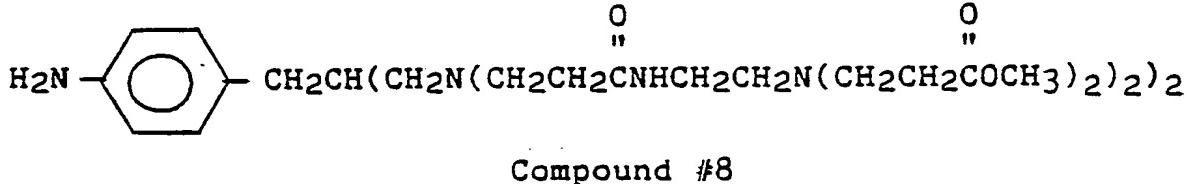
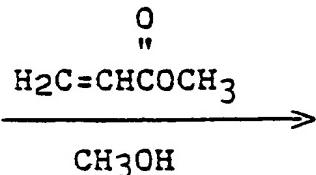
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Example L: Preparation of a starburst polymer (containing an aniline moiety) of one and one half generations; represent by the following scheme:

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Compound #7

+



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The amine (Compound #7) (1.45 g, trace of methanol remained) was dissolved in methanol (100 ml) and was added slowly over 1½ hours to a stirred solution of methyl acrylate (5.80 g) in methanol (20 ml). The solution was allowed to stir for 24 hours at room temperature. Removal of the solvent followed by repeated azeotroping with methanol enabled the removal of all the excess methyl acrylate. After pumping down on a vacuum pump for 48 hours the ester (Compound #8) was isolated as an orange oil (2.50 g, 1.8 mmole).

Example M: Hydrolysis of 4.5 generation dendrimer and preparation of calcium salt.

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4.5 Generation PAMAM (ester terminated, initiated off NH₃) (2.11 g, 10.92 meq) was dissolved in 25 ml of methanol and to it was added 10% NaOH (4.37 ml, 10.92 meq) (pH = 11.5-12). After 24 hours at room temperature, the pH was about 9.5. After an additional

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20 hours, the solution was rotovaped, 50 ml of toluene added, and rotovaped again.

The resulting oil was dissolved in 25 ml of methanol and precipitated as a white gum upon addition of 75 ml of diethyl ether. The liquid was decanted and the gum was rotovaped to give a very fine off-white powder which upon drying gives 2.16 g of product (98% yield). No ester groups were found upon NMR and infrared analysis.

The sodium salt of 4.5 Generation PAMAM (ester terminated, initiated from NH₃) was replaced by the calcium salt via dialysis. The sodium salt (1.03 g) was dissolved in 100 ml of water and passed through hollow fiber dialysis tubing (cut off = 5000) at 3 ml/minute. The exterior of the tubing was bathed in 5% CaCl₂ solution. This procedure was then repeated.

The resulting solution was again dialyzed, this time against water, then repeated two additional times.

Evaporation provided 0.6 g of wet solid, which was taken up in methanol (not totally soluble) and is dried to give 0.45 g of off-white crystals.

C₃₆H₅₉Ca₂₄N₉ Calc. - 10.10% Ca⁺⁺

M Wt. = 9526.3 Calc. = C-4432.1, H-601.8, O-2255.9,
N-1274.6, Ca-961.9)

Theo: C-46.5, H-6.32, N-13.38, Ca-10.10
Found: C-47.34, H-7.00, N-13.55, Ca-8.83

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Example N: Preparation of dendrimers with terminal carboxylate groups.

Half-generation starburst polyamidoamines were hydrolyzed to convert their terminal methyl ester groups to carboxylates. This generated spheroidal molecules with negative charges dispersed on the periphery. The dendrimers hydrolyzed ranged from 0.5 generation (three carboxylates) to 6.5 generation (192 carboxylates).

The products could be generated as Na^+ , K^+ , Cs^+ or Rb^+ salts.

Example O: N-t-butoxycarbonyl-4-aminobenzyl malonate dimethylester

4-Aminobenzyl malonate dimethylester (11.62 g, 49 mmol) was dissolved in 50 ml of t-butanol:water (60:40 with stirring. Di-t-butoxydicarbonate (19.79 g, 90 mmol) was added and the reaction mixture stirred overnight. The butanol was removed on the rotary evaporator, resulting in a yellow suspension of the product in water. Extraction into methylene chloride, drying (MgSO_4) and evaporation gave a yellow oil (21.05 g, contaminated by di-t-butoxydicarbonate).

Recrystallization from 2-propanol:water (75:25) yield pale yellow crystals (11.1 g, 33 mmol, 67%). The structure was confirmed by ^{13}C NMR and purity checked by hplc analysis (spherisorb ODS-1, 0.05M H_3PO_4 pH 3: CH_3CN 55:45). The material was used without further purification.

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Example P: N-t-butoxycarbonyl-6-(4-aminobenzyl)-
1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-4-aminobenzyl malonate dimethylester (8.82 g 26 mmol), prepared in Example 0, 5 was dissolved in 50 ml of methanol. This solution was added dropwise (2 hours) to a solution of freshly distilled ethylenediamine (188 g 3.13 mole) and 20 ml of methanol, under a nitrogen atmosphere. The solution was allowed to stir for 24 hours. The ethylene 10 diamine/methanol solution was removed on the rotary evaporator. The product was dissolved in methanol and toluene added. Solvent removal on the rotary evaporator gave the crude product as a white solid (10.70 g contaminated with ethylenediamine). The 15 sample was divided into two samples for purification. Azeotropic removal of ethylenediamine with toluene, using a soxhlet extractor with sulphonated ion exchange beads in the thimble to trap the ethylenediamine, 20 resulted in partial decomposition of the product, giving a brown oil. The remaining product was isolated as a white solid from the toluene on cooling (2.3 g approximately 50 percent). Analysis of a 10 percent solution in methanol by gas chromatography (Column, 25 Tenax 60/80) showed no ethylenediamine detectable in the sample (<0.1 percent). The second fraction was dissolved in methanol to give a 10 percent solution (by weight) and purified from the ethylenediamine by 30 reverse osmosis, using methanol as the solvent. (The membrane used was a Filmtec FT-30 , in an Amicon TC1R thin channel separator, the ethylenediamine crossing the membrane.) The product was isolated as a white solid (2.7 g), in which no detectable amounts of 35 ethylenediamine could be found by gas chromatography. The ¹³C NMR data and HPLC analysis (Spherisorb ODS-1,

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0.05M H₃PO₄ pH 3:CH₃CN 55:45) were consistent with the proposed structure. The product was used with no further purification.

5 Example Q: Preparation of a starburst dendrimer of one half generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (5.0 g 13 mmol), prepared in
10: Example P, was dissolved in 100 ml of methanol. Methyl acrylate (6.12 g, 68 mmol) was added and the solution stirred at ambient temperatures for 72 hours. The reaction was monitored by HPLC (Spherisorb ODS1,
15 Acetonitrile: 0.04M Ammonium acetate 40:60) to optimize conversion to the desired product. The solution was concentrated to 30 percent solids, and methyl acrylate (3.0 g 32 mmol) was added. The reaction mixture was stirred at ambient temperatures until no partially
20 alkylated products were detectable by HPLC (24 hours). Removal of the solvent at 30°C by rotary evaporation, and pumping down at 1 mm Hg for 24 hours gave the product as yellow viscous oil, yield 7.81 g. The ¹³C NMR data was consistent with the proposed structure.
25 The product was used without further purification.

Example R: Preparation of a starburst dendrimer of one full generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

30 The half generation product (Example Q) (7.70 g, 10.45 mmol) was dissolved in 75 ml of methanol and added dropwise over 2 hours to a stirred solution of ethylenediamine (400 ml, 7.41 mol) and methanol (50 ml). The reaction mixture was stirred at ambient temperatures for 48 hours. The ethylenediamine and

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methanol were removed by rotary evaporation to give a yellow oil (11.8 g contaminated with ethylenediamine). The product was dissolved in 90 ml of methanol, and purified from the ethylenediamine by reverse osmosis
5 (Filmtec FT-30 membrane and Amicon TC1R thin channel separator, methanol as solvent). After 48 hours, no ethylenediamine could be detected by gas chromatography (Column, Tenax 60/80). Removal of the solvent on the rotary evaporator, followed by pumping down on a vacuum
10 line for 24 hours gave the product as a yellow glassy solid (6.72 g). Analysis by HPLC, PLRP-S column, acetonitrile:0.015M NaOH, 10-20 percent gradient in 20 min.) and ^{13}C NMR analysis was consistent with the
15 proposed structure.

Example S: Preparation of a starburst polymer of one and one half generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

20 The one generation product (Example R) (2.14^o g, 25 mmol) was dissolved in 12.5 ml of methanol, and methyl acrylate (3.5 g, 39 mmol) in 5 ml of methanol was added. The solution was stirred at ambient temperatures for 48 hours, monitoring the progress of
25 the reaction by HPLC (Spherisorb ODS-1, acetonitrile: 0.04M ammonium acetate, 60:40). A second aliquot of methyl acrylate was added (3.5 g 39 mmol) and the reaction mixture stirred at ambient temperatures for a further 72 hours. Removal of the solvent on the rotary
30 evaporator gave the product as a yellow oil (3.9 g) after pumping down overnight with a vacuum pump. The product was used with no further purification.

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Example T: Preparation of a starburst polymer of two full generations from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

The one and one half generation product (Example S) (3.9 g, 2.5 mmol) was dissolved in 50 ml of methanol, and was added dropwise over 2 hours to a stirred solution of ethylenediamine (600 g, 10 mol) and methanol (50 ml). The solution was stirred at ambient temperatures under an atmosphere of nitrogen for 96 hours. The ethylenediamine/methanol was removed on the rotary evaporator to give a yellow glassy solid (4.4 g contaminated with ethylenediamine). A 10 percent solution of the product was made in methanol, and purified from the ethylenediamine by reverse osmosis (membrane used as a Filmtec FT-30, in an Amicon TC1R thin channel separator) until no ethylenediamine could be detected by gas chromatography (Column, Tenax 60/80. Removal of the solvent gave the product as a yellow glassy solid (3.52 g). The ^{13}C NMR data and HPLC analysis (PLRP-S column, acetonitrile:0.015 M NaOH, 10 to 20 percent gradient in 20 minutes, were consistent with the proposed structure.

Example U: Reaction of the two generation starburst with Bromoacetic Acid to give a methylene carboxylate terminated starburst dendrimer

The second generation product (Example T) (0.22 g, 0.13 mmol) was dissolved in 15 ml of deionized water and the temperature equilibrated at 40.5°C. Bromoacetic acid (0.48 g, 3.5 mmol) and lithium hydroxide (0.13 g, 3.3 mmol) were dissolved in 5 ml of deionized water, and added to the reaction mixture. The reaction pH was carefully maintained at 9, with the use of a pH stat (titrating with 0.1N NaOH), at 40.5°C overnight.

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Monitoring by reverse phase HPLC, (Spherisorb ODS-1 column, eluent 0.25 M H_3PO_4 pH 3 [NaOH]; acetonitrile 85:15) confirmed the synthesis of predominantly a single component.

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Example V: Preparation of Isothiocyanato functionalized second generation methylene-carboxylate terminated starburst dendrimer

10 Five ml of a 2.8 mM solution of the second generation methylenecarboxylate terminated starburst dendrimer (Example U) was diluted with 20 ml water and the pH adjusted to 0.5 with concentrated hydrochloric acid. After one hour at room temperature the mixture was analyzed by HPLC to verify the removal of the 15 butoxycarbonyl group and then treated with 50 percent sodium hydroxide to bring the pH to 7. A pH stat (titrating with 0.1 N NaOH) was used to maintain the pH at 7 and 225 μ l thiophosgene was added. After 15 minutes at room temperature the pH of the mixture was 20 adjusted to 5 with 1N HCl. The mixture washed with chloroform (20 ml x 2) then concentrated on a rotary evaporator at reduced pressure. The residue recovered 25 0.91 g is a mixture of the isothiocyanate and salts.

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Example W: Preparation of second generation starburst polyethyleneimine-methane sulfonamide

30 To a solution of 125 g N-methanesulfonyl-aziridine in 50 ml ethanol was added 25.0 g tris(2-aminoethyl)amine. The solution was stirred at room temperature for 4 days. Water was added to the reaction mixture as needed to maintain the homogeneity of the solution. The solvent was removed by 35 distillation in vacuo to give the 2nd generation

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starburst PEI-methane sulfonamide as a yellow glass (161 g).

5 Example X: Cleavage of methane sulfonamides to form second generation starburst polyethyleneimine

A solution of 5.0 g of second generation starburst PEI-methane sulfonamide, from Example W in 20 ml of 38 percent HCL was sealed in a glass ampoule. 10 The ampoule was heated at 160°C for 16 hours, then cooled in an ice bath and opened. The solvent was removed by distillation in vacuo and the residue dissolved in water. After adjusting the pH of the solution to greater than or equal to 10 with 50 percent 15 NaOH, the solvent was removed by distillation in vacuo. Toluene (150 ml) was added to the residue and the mixture heated at reflux under a Dean-Stark trap until no more water could be removed. The solution was filtered to remove salts and the filtrate concentrated 20 in vacuo to give 1.9 g second generation starburst PEI as a yellow oil.

25 Example Y: Preparation of third generation starburst polyethyleneimine-methane sulfonamide

To a solution of 10.1 g second generation starburst PEI, from Example X, in 100 ml ethanol was added 36.6 g N-methanesulfonylaziridine. The solution was stirred at room temperature for 1 week. Water was 30 added as needed to maintain the homogeneity of the solution. The solvent was removed by distillation in vacuo to give third generation starburst PEI-methane sulfonamide as a yellow glass (45.3 g).

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Example Z: Cleavage of methane sulfonamides to form 3rd
gen starburst polyethyleneimine

The methane sulfonamide groups of third
5 generation starburst PEI-methane sulfonamide (5.0 g),
from Example Y, were removed by the same procedure as
described for the second generation material in Example
X to give 2.3 g third generation starburst PEI as a
10 yellow oil.

Example AA: Preparation of a methylenecarboxylate
terminated second generation starburst polyamidoamine
(initiated from ammonia)

15 The second generation starburst polyamidoamine
(2.71 g, 2.6 mmol) and bromoacetic acid (4.39 g, 31.6
mmol) were dissolved in 30 ml of deionized water and
the pH adjusted to 9.7 with 5N NaOH using a pH stat.
The reaction was maintained at this pH for a half hour,
20 and the temperature was slowly raised to 60°C and was
maintained at 60°C for three hours at constant pH. The
pH was raised to 10.3, and the reaction mixture
remained under control of the pH stat at ambient
temperatures overnight. The reaction mixture was
25 refluxed for a further four hours prior to work up.
Removal of the solvent, and azeotroping the final
traces of water with methanol gave the product as a
pale yellow powder (8.7 g, contaminated with sodium
bromide). The ¹³C NMR spectrum was consistent with the
30 proposed structure (with some contamination due to a
small amount of defected material as a result of some
monoalkylation).

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Example BB: Preparation of a methylenecarboxylate terminated second generation starburst polyethyleneimine (initiated from ammonia)

The second generation starburst polyethyleneimine (2.73 g, 6.7 mmol), from Example X, and bromoacetic acid (11.29 g 81 mmol) were dissolved in 30 ml of deionized water. The pH was slowly raised to pH 9.5 maintaining the temperature below 30°C. The temperature was raised slowly to 55°C, and the reaction pH maintained at 9.5 for 6 hours with the aid of a pH stat (titrating with 5N NaOH). The pH was raised to 10.2, and maintained at that pH overnight. Removal of the solvent on the rotary evaporator, and azeotroping the final traces of water using methanol, gave the product as a yellow powder (17.9 g, contaminated with sodium bromide). The ^{13}C NMR spectrum was consistent with the proposed structure (with some contamination due to a small amount of defected material as a result of some monoalkylation).

Example CC: Preparation of a 3.5, 4.5, 4.4 and 6.5 generation starburst PAMAM:

To a 10 weight percent methanolic solution of 2.46 g 3 generation PAMAM starburst was added 2.32 g of methyl acrylate. This mixture was allowed to sit at room temperature of 64 hours. After solvent and excess methyl acrylate removal, 4.82 g of product was recovered (105 percent of theoretical).

Preparation of higher 1/2 generation starburst PAMAM's:

Generations 4.5, 5.5 and 6.5 were prepared as described above with no significant differences in

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reactant concentrations, reactant mole ratios or reaction times.

Example DD: Preparation of 4, 5 and 6 generation starburst PAMAM:

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To 2000 g of predistilled ethylenediamine was added 5.4 g of 4.5 generation starburst PAMAM as a 15 weight percent solution in methanol. This was allowed to sit at room temperature for 48 hours. The methanol and most of the excess ethylenediamine were removed by rotary evaporation under water aspirator vacuum at temperature less than 60°C. The total weight of product recovered was 8.07g. Gas chromatography indicated that the product still contained 34 weight percent ethylenediamine at this point. A 5.94 g portion of this product was dissolved in 100 ml methanol and ultrafiltered to remove the residual ethylenediamine. The filtration was run using an Amicon TC1R thin channel recirculating separator equipped with an Amicon YM2 membrane. An in-line pressure relief valve was used to maintain 55 psig (380 kPa) pressure across the membrane. The 100 ml was first concentrated to 15 ml by forcing solvent flow exclusively through the membrane. After this initial concentration, the flow was converted to a constant volume retentate recycle mode for 18 hours. After this time, 60 ml of methanol was passed over the membrane to recover product still in the module and associated tubing. The product was stripped of solvent and 2.53 g of 5 generation starburst PAMAM was recovered. Analysis by gas chromatography indicated 0.3 percent residual ethylenediamine remained in the product.

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Preparation of generation 4 and 6 proceeded as above with the only difference being the weight ratio of ethylenediamine to starting material. To prepare 4th generation this ratio was 200:1 and for 6th 5 generation this ratio was 730:1.

Example 1: Preparation of a product containing more than one rhodium atom per starburst polymer.

2.5 Generation PAMAM (ester terminated, 10 initiated from NH₃) (0.18 g, 0.087 mmole) and RhCl₃·3H₂O (0.09 g, 0.3 mmole) were mixed in dimethylformamide (DMF) (15 ml) and heated for 4 hours at 70°C. The solution turned crimson and most of the rhodium was taken up. The unreacted rhodium was 15 removed by filtration and the solvent removed on the rotary evaporator. The oil formed was chloroform soluble. This was washed with water and dried (MgSO₄) before removal of solvent to yield a red oil (0.18 g). 20 The NMR spectrum was recorded in CDCl₃ only minor differences were noted between the chelated and unchelated starburst. Dilution of some of this CDCl₃ solution with ethanol followed by NaBH₄ addition resulted in rhodium precipitation. RhCl₃·3H₂O is 25 insoluble in chloroform and in chloroform starburst solution thus confirming chelation.

Example 2: Preparation of a product containing Pd chelated to starburst polymer.

30 3.5 Generation PAMAM (ester terminated, initiated from NH₃) (1.1 g, 0.24 mmole) was dissolved with stirring into acetonitrile (50 ml). Palladium chloride (0.24 g, 1.4 mmole) was added and the solution 35 was heated at 70-75°C (water bath) overnight. The PdCl₂ was taken up into the starburst. After removal of the

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solvent, the NMR in CDCl_3 confirmed that chelation had occurred. Dilution of the CDCl_3 solution with ethanol and addition of NaBH_4 resulted in precipitation of the palladium. The chelated product (1.23 g) was isolated
5 as a brown oil.

Example 3 : Attachment of herbicidal molecules (2,4-D)
to the surface of starburst dendrimers.

10 Third generation PAMAM (initiator core= NH_3) (2.0 g, 0.8 mmole) was dissolved in H_2O (10 ml) and combined with toluene (20 ml). The two-phase system was then stirred and cooled with an ice bath at which time the acid chloride of 2,4-D [2,4-dichlorophenoxy-acetic acid] (2.4 g, 12 equiv) dissolved in toluene (10 ml) was added dropwise over 30 minutes. When the addition was nearly complete; NaOH (0.5 g, 12.5 mmole, 50% w/w solution) was added and the solution stirred for an additional two hours. The reaction mixture was
15 then evaporated to dryness and the resulting solid residue repeatedly taken up in $\text{CHCl}_3/\text{MeOH}$ (1:1) and filtered. The tan solid was not totally soluble in CHCl_3 and appeared to be insoluble in water; however, the addition of acetone facilitated dissolution. The
20 tan solid was stirred in CHCl_3 for 24 hours and the solution filtered (a sticky tan solid was obtained). After drying over MgSO_4 , the filtrate was concentrated to give a viscous orange oil which solidified on
25 standing. The ^{13}C NMR partial amidation at the surface by 2,4-D is consistent with the association of the 2,4-D to starburst dendrimer.
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Example 4: Incorporation of 2,4-dichlorophenoxyacetic acid (2,4-D) into starburst dendrimers.

A widely accepted method for ascertaining whether a "probe molecule" is included in the interior of a micelle is to compare its carbon-13-spin lattice relaxation times (T_1) in a non-micellized versus micellized medium. A substantial decrease in T_1 for the micellized medium is indicative of "probe molecule" inclusion in the micelle. Since starburst dendrimers are "covalently fixed" analogs of micelles, this T_1 relaxation time technique was used to ascertain the degree/extent to which various herbicide type molecules were associated with starburst polyamidoamines. In the following examples, T_1 values for 2,4-dichlorophenoxy-acetic acid (I) (2,4-D) were determined in solvent ($CDCl_3$) and then compared to T_1 values in $CDCl_3$ at various [I:dendrimer] molar ratios.

Inclusion of 2,4-D into various starburst polyamido-amine dendrimers as a function of generation.

Various half generation (ester terminated, initiated from NH_3) starburst polyamidoamine dendrimers (Generation (Gen) = 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5) were combined with 2,4-dichlorophenoxyacetic acid (I) in $CDCl_3$ to give an acid:tertiary amine ratio of 1:3.5 and molar ratios of acid:dendrimer of 1:86 as shown in Table III. The relaxation times (T_1) obtained for the various carbon atoms in 2,4-dichlorophenoxyacetic acid and a generation = 3.5 starburst PAMAM dendrimers are shown in Table IV, both for 1:1 acid/amine ratios and for saturated solutions of 2,4-D.

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Table III

<u>5</u>	<u>Gen</u>	<u>(A)</u> Acid/Amine	<u>(B)</u> Acid/Amine	<u>(C)</u> Acid/Total Nitrogen	<u>(D)</u> Molar Ratio (Acid/Star- burst)
	0.5	1	--	1	1
	1.5	1	1.33	0.57	6
10	2.5	1 (3.5)*	1.11 (3.8)*	0.53 (1.8)*	9 (34)*
	3.5	1 (3.0)*	1.05 (3.2)*	0.51 (1.6)*	20 (67)*
	4.5	1	1.02	0.51	42
	5.5	1	1.01	0.50	86

* represents examples of 2,4-D inclusion into the interior of the dendrimer in amounts greater than stoichiometric.

Table IV
T₁'s for 2,4-D/G = 3.5 PAMAM Starburst
Inclusion complex: Concentration Effects

<u>Carbon</u>	<u>(A)</u>	<u>(B)</u>		
	<u>1:1 Acid/Amine</u>	<u>Saturated with 2,4-D</u>		
	T ₁	13C**	T ₁	13C**
1	3.19±.12	(152.73)	3.08±.09	(152.30)
3	0.34±.01	(128.64)	0.29±.01	(129.62)
5	0.38±.01	(127.41)	0.32±.01	(127.34)
2	3.28±.08	(125.79)	2.72±.68	(125.99)
4	4.58±.16	(123.27)	3.95±.07	(123.16)
6	0.31±.01	(114.66)	0.28±.01	(114.48)
CH ₂	0.16±.01	(67.29)	0.146±.003	(66.79)
C=O	1.24±.07	(170.12)	-	-

** represents 13C chemical shifts referenced to chloroform at 76.9 ppm..

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These data show that larger than stoichiometric amounts of 2,4-dichlorophenoxyacetic acid (i.e., [(I):Gen=3.5 dendrimer]) = 67 can be used without increasing the T_1 in any case in the saturated state (see Columns (A) and 5 (B) in Table IV). In fact, the relaxation times T_1 (Column (B) are decreased slightly, thus indicating that larger than stoichiometric amounts of 2,4- dichlorophenoxyacetic acid can be included into the 10 interior of the dendrimer. For example, a molar ratio of

[(I):Gen=2.5 dendrimer] = 34 whereas [(I):Gen=3.5 dendrimer] = 67,

15 (see Column D in Table III).

Figure 3 is a plot of T_1 values for carbons-3, 5 and 6 in 2,4-dichlorophenoxyacetic acid as a function of dendrimer generation (i.e., 0.5 → 5.5). A minimum 20 in T_1 is reached in all cases of generation 2.5 → 5.5, thus indicating incorporation in that dendrimer generation range is occurring. Figure 3 also includes T_1 values for 2,4-D in the presence of triethylamine [N(Et)₃] and N(Et)₃ + N-methylacetamide. It can be seen 25 that these values are much larger than for dendrimers G = 1.5 → 5.5, thus further supporting molecular incorporation into the dendrimer molecule.

30 Example 5: Demonstration of multiple chelation of yttrium by a methylene carboxylate terminated second generation starburst polyethyleneimine by trans chelation from yttrium acetate

The starburst polyethyleneimine methylene carboxylate terminated material (0.46 g 52.5 percent 35 active, remainder sodium bromide, 0.18 mmol active starburst dendrimer), from Example BB, was dissolved in

4.5 ml of deuterium oxide. The resultant pH was 11.5-12. A solution of yttrium acetate was prepared by dissolving yttrium chloride (0.15 g, 0.5 mmol) and sodium acetate (0.41 g, 0.5 mmol) in 1.5 ml of deuterium oxide (2.9 moles of yttrium per mole of dendrimer). Aliquots of 0.5 ml of the yttrium acetate solution were added to the dendrimer solution and the ^{13}C NMR spectra recorded at 75.5 MHz.

The ^{13}C NMR spectrum of yttrium acetate shows two resonances, 184.7 ppm for the carboxyl carbon and 23.7 ppm for the methyl carbon, compared with 182.1 and 24.1 ppm for sodium acetate, and 177.7 and 20.7 ppm for acetic acid (Sadler ^{13}C NMR Standard Spectra).

Monitoring the positions of these bands indicates degree of chelation with the starburst dendrimer. The most informative signal for the starburst dendrimer which is indicative of chelation is the $\alpha\text{-CH}_2$ (of the methylene carboxylate group involved in chelation), which appears at 58.4 ppm in the unchelated dendrimer, and 63.8 ppm in the chelated dendrimer. Upon chelation with yttrium, the spin lattice relaxation times of the time $\alpha\text{-CH}_2$ shortens as expected from $0.24 \pm 0.01\text{s}$ to $0.14 \pm 0.01\text{s}$, indicative of chelation.

Following the addition of 0.5 ml of the yttrium acetate solution to the starburst dendrimer, all the yttrium appeared to be chelated by the dendrimer, confirmed by the signals for the acetate being that of sodium acetate. The same observation was noted for the addition of a second 0.5 ml aliquot of the yttrium acetate solution. Upon addition of the third aliquot of yttrium acetate, not all of the yttrium was observed

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to be taken up as the starburst chelate, the acetate carboxyl resonance was observed to shift to 183.8 ppm indicating that some of the yttrium was associated with the acetate. The integrated area of the chelated -CH₂ groups on the dendrimer increased, indicating that some of the third mole equivalent of yttrium added was indeed chelated with the dendrimer. These results indicate that the dendrimer can chelate from 2-3 yttrium ions per dendrimer molecule.

10.

Example 6: Demonstration of Multiple Chelation of Yttrium by a methylene carboxylate terminated second generation starburst polyamidoamine by trans chelation from yttrium acetate.

15

The same experimental methods were used for this study as were used for Example 5. The starburst polyamidoamine methylene-carboxylate terminated material (0.40g 62.5% active, remainder sodium bromide, 0.12 mmol.) was dissolved in 4-5 ml of deuterium oxide. 20 The resultant pH was 11.5-12, which was lowered to 9.4 with 6N HCl prior to the experiment. A solution of yttrium acetate was prepared by dissolving yttrium chloride (0.1125g, .37 mmol.) and sodium acetate (0.0915g, 1.1 mmol.) in 1.5 ml of deuterium oxide, thus 25 every 0.5 ml of solution contains one mole equivalent of metal.

30 The first two mole equivalents of yttrium acetate added were fully chelated by the starburst polyamidoamine. On addition of a third mole equivalent of yttrium, precipitation of the product occurred and as such no NMR data could be obtained. The signals 35 which gave the most information about chelation by the starburst dendrimer were those of the two carbons adjacent to the chelating nitrogen. The chemical

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shifts of these carbons in the unchelated dendrimer occurred at 59.1 ppm for the α -CH₂, and 53.7 ppm for the first methylene carbon of the backbone. Upon chelation these two resonances were observed to shift
5 downfield to 60.8 and 55.1 ppm respectively. The trans chelation shows that two metal ions can be readily chelated per dendrimer molecule, however upon chelation of some unknown fraction of a third mole equivalent, the product precipitates out of solution.

10

Example 7: Demonstration of Multiple Chelation of ⁹⁰Y by a methylenecarboxylate terminated second generation starburst polyethyleneimine.

15 Standard solution of yttrium chloride (3×10^{-2} M, spiked with non-carrier added ⁹⁰Y) and methylenecarboxylate terminated second generation starburst polyethyleneimine (6×10^{-2} M) were prepared. These were reacted together at various metal:starburst
20 ratios in HEPES buffer. The complex yield was determined by ion exchange chromatography using Sephadex G50 ion exchange beads, eluting with 10% NaCl:NH₄OH, 4:1 at pH 10. Noncomplexed metal is removed on the column, complexed metal elutes. Yields
25 were obtained by comparing the radioactivity eluted with that on the column, using a well counter.

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Table V

Chelation of 2.5 Gen. PEI Acetate with ^{90}Y

5

	<u>Vol. Y+3</u>	<u>Vol. PEI</u>	<u>Vol HEPES</u>	<u>M:L Theor.</u>	<u>% Complex</u>	<u>M:L Act.</u>
	5	30	370	0.1	110	0.1
	10	30	360	0.2	101	0.2
10	20	30	350	0.4	95	0.4
	30	35	340	0.5	97	0.5
	30	30	340	0.5	102	0.5
	60	30	310	1.0	99	1.0
15	120	30	250	2.0	100	2.0
	180	30	180	3.0	94	2.8
	250	30	120	4.1	80	3.3
	300	20	80	7.5	44	3.3
20	300	20	70	5.0	40	2.0
	300	20	70	5.0	41	2.0

All volumes in Table V are in microlitres

Within the accuracy of the experiments, these results
 25 indicate that the 2.5 Gen. starburst PEI acetate can
 chelate between 2 and 3 metals per polymer giving a
 soluble complex.

30 Example 8: Demonstration of multiple chelation of iron
 by a sodium propionate terminated sixth generation
 starburst polyamidoamine.

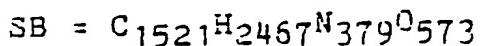
The sodium propionate terminated sixth
 generation polyamidoamine (initiated from ammonia)
 35 (97.1 mg, 2.45 mol.) was dissolved in 1.5 ml of
 deionized water. Addition of 0.5 ml of 0.5N HCl

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reduced the pH to 6.3. Ferric chloride was added (0.5 ml of 0.12M solution, 0.051 mmol) producing a light brown gelatinous precipitate. On heating at 60°C for 0.5 hours, the gelatinous precipitate became soluble, 5 resulting in a homogeneous orange solution. The solution was filtered through Biogel P2 acrylamide gel (10 g, twice) isolating the orange band (free of halide contamination). Removal of the solvent in vacuo gave the product as an orange film (30 mg). Analysis was 10 consistent with chelation of approximately 20 moles of ferric ions per mole of starburst dendrimer.

Table IV

15	Found	Theoretical		
		$\text{Na}_4\text{Fe}_{20}\text{H}_{128}\text{SB}$	$\text{Na}_5\text{Fe}_{20}\text{H}_{127}\text{SB}$	$\text{Na}_6\text{Fe}_{20}\text{H}_{126}\text{SB}$
	Na 0.39, 0.24 (0.31 0.18)	0.25	0.31	0.38
20	Fe 3.14, 3.11 (3.12 0.028)	3.05	3.05	3.04
	C 47.11	49.87	49.84	49.81
	H 7.33	7.31	7.30	7.29
	N 14.81	14.49	14.48	14.47
25	O ----	25.03	25.02	25.01
	Mwt.	36632.23	36654.21	36375.13



30 These results confirm chelation of 20±2 moles of ferric ions per mole of starburst dendrimer.

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WE Claim:

1. A starburst conjugate which comprises at least one starburst polymer associated with at least one unit of at least one carried agricultural material.

2. The conjugate of Claim 1 wherein the starburst polymer is a starburst dendrimer.

5

3. The conjugate of Claim 1 or 2 wherein at least one of the carried agricultural materials is a pesticide, radionuclide, chelant, toxin, signal generator, signal reflector, or signal absorber.

10

4. The conjugate of Claim 2 wherein there are at least two different carried materials at least one of which is a target director and at least one of which is a bioactive agent.

15

5. The conjugate of Claim 4 wherein the target director is an entity specific for one or more target receptors and the bioactive agent is a radionuclide, pesticide or toxin.

20

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6. The conjugate of Claim 1 wherein the dendrimer contains discontinuities.

7. A starburst conjugate of claim 1 the
5 formula:

$$(P)_x * (M)_y \quad (I)$$

wherein each P represents a dendrimer;

10 x represents an integer of 1 or greater;

each M represents a unit of a carried agricultural material, said carried agricultural material can be the same carried agricultural material or a different
15 carried agricultural material;

y represents an integer of 1 or greater; and

* indicates that the carried agricultural material is
20 associated with the dendrimer.

8. The conjugate of Claim 7 wherein M is a pesticide, radionuclide, chelator, chelated metal,
25 toxin, signal generator, signal reflector, or signal absorber.

9. The conjugate of Claim 7 wherein x=1 and
y=2 or more.

30

10. The conjugate of Claim 8 wherein y=2 or
more.

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11. The starburst conjugate of Claim 7 wherein
the molar ratio of any ionic M to P is 0.1-1,000:1.

5 12. The starburst conjugate of Claim 9 wherein
the weight of the ratio of any pesticide or toxin M to
P is 0.1-5:1.

10 13. A process for preparing

(P)_x * (M)_y (I)

15 wherein each P represents a dendrimer; x represents an
integer of 1 or greater; each M represents a unit of a
carried agricultural material, said carried
agricultural material can be the same carried
agricultural material or a different carried
20 agricultural material; y represents an integer of 1 or
greater; and * indicates that the carried agricultural
material is associated with the dendrimer, which
comprises reacting P with M, usually in a suitable
solvent, at a temperature which facilitates the
25 association of the carried agricultural material (M)
with the starburst dendrimer (P).

30 14. The process of Claim 13 wherein the
temperature is from room temperature to reflux.

35 15. The process of Claim 13 wherein the
suitable solvent is water, methanol, ethanol,
chloroform, acetonitrile, toluene, dimethylsulfoxide or
dimethylformamide.

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16. A starburst conjugate composition which comprises one or more starburst conjugates of any one of Claims 1-12 and at least one agriculturally acceptable diluent or carrier.

5

17. The starburst conjugate composition of Claim 16 which also contains other active ingredients.

10

18. A starburst conjugate composition of any one of Claims 1-12, 16 or 17 for use as an in vivo or in vitro diagnostic compound.

15

19. A starburst conjugate composition of any one of Claims 1-12, 16 or 17 for use as an in vivo therapeutic compound.

20

20. A method for the delivery of at least one carried agricultural material which comprises administering of at least one starburst conjugate, as defined in Claims 1-12, 16 or 17 containing said material at or near a targeted locus.

25

21. A method for scavenging therapeutic or diagnostic compounds which comprises in vivo administration of a bifunctional starburst conjugate of any one of Claims 1-12, 16 or 17 containing a target director which localizes the conjugate to a target locus and a scavenging moiety which can bind a secondarily administered therapeutic or diagnostic compound.

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22. The method of Claim 21 wherein the scavenging moiety is a chelant.

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- 75 -

AMENDED CLAIMS

[received by the International Bureau on 12 January 1988 (12.01.88)
original claim 3 amended; new claims 23 - 29 added ; other claims unchanged (5 pages)]

1. A starburst conjugate which comprises at least one starburst polymer associated with at least one unit of at least one carried agricultural material.

2. The conjugate of Claim 1 wherein the starburst polymer is a starburst dendrimer.

5 3. The conjugate of Claim 1 or 2 wherein at least one of the carried agricultural materials is a pesticide, radionuclide, chelator, chelated metal, toxin, signal generator, signal reflector, or signal absorber.

10 4. The conjugate of Claim 2 wherein there are at least two different carried materials at least one of which is a target director and at least one of which is a bioactive agent.

15 5. The conjugate of Claim 4 wherein the target director is an entity specific for one or more target receptors and the bioactive agent is a radionuclide, pesticide or toxin.

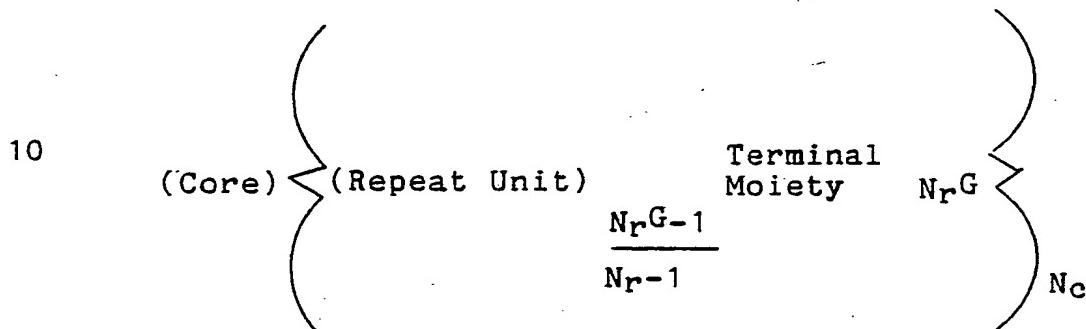
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22. The method of Claim 21 wherein the scavenging moiety is a chelant.

5 23. The conjugate of Claim 2 wherein the starburst dendrimer is of the formula



15 wherein: the core is

of terminal groups per dendritic branch =

$$20 \quad \frac{\text{Nr}^G}{2};$$

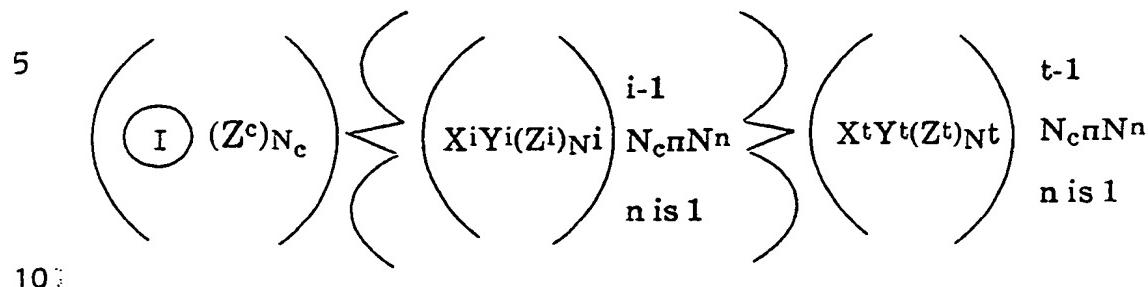
25 G is the number of generations; Nr is the repeating unit multiplicity which is at least 2; Nc is the valency of the core compound; the terminal moiety is determined by the following:

of terminal moieties per dendrimer =

$$30 \quad \frac{N_c N_r^G}{2}$$

35 wherein Nr, G and Nc are as defined above; and the Repeat Unit has a valency or functionality of Nr + 1 wherein Nr is as defined above.

24. The conjugate of Claim 2 wherein the starburst dendrimer is of the formula



wherein i is 1 to $t-1$; the core compound is represented by the formula

15 (Z^c)_{N_c}

where

25. and N_c represents the core valency; the repeat unit is represented by the formula $X_i Y_i (Z_i)_{N_i}$ wherein "i" is defined as above; the final or terminal units are represented by $X_t Y_t (Z_t)_{N_t}$ wherein t represents terminal generation and X_t , Y_t , Z_t and N_t may be the same as or different from X_i , Y_i , Z_i and N_i except that there is no succeeding generation connected to the Z_t groups and N_t may be less than two; the n function is the product of all the values between its defined limits, such as

i - 1

$$\Pi \quad N_n = (N_1)(N_2)(N_3) \dots (N_{i-2})(N_{i-1})$$

5

n=1

which is the number of repeat units, $X_i Y_i (Z_i)^{N_i}$, comprising the i th generation of one dendritic branch and when i is 1, then $n^o = 1$.

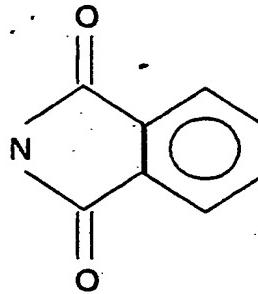
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n=1

25. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, with an

15 aniline moiety, which may have the NH₂ group protected by an N-phthalimide of the formula

20



25

26. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, which may have the NH₂ group protected by any protecting group used for amines which is inert under the conditions used for 30 starburst synthesis.

30

27. A process for preparing a starburst polyethyleneimine which comprises reacting a starburst polyethyleneiminemethane sulfonamide with hydrochloric acid.

35

28. A process for purifying a starburst dendrimer having a solvent present which comprises removing the solvent by ultrafiltration using a membrane.

5

29. The process of Claim 28 wherein the solvent is ethylenediamine.

10

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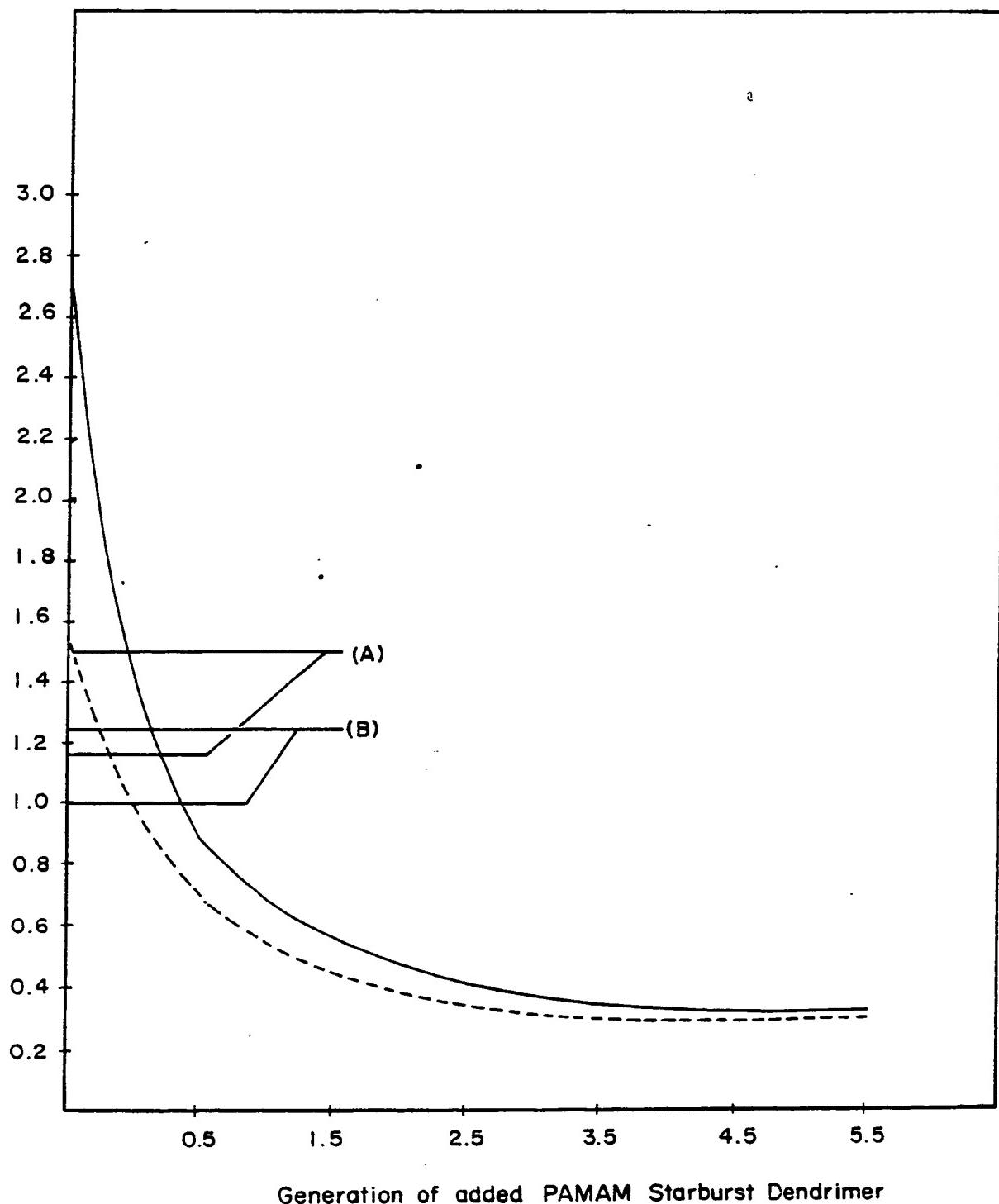
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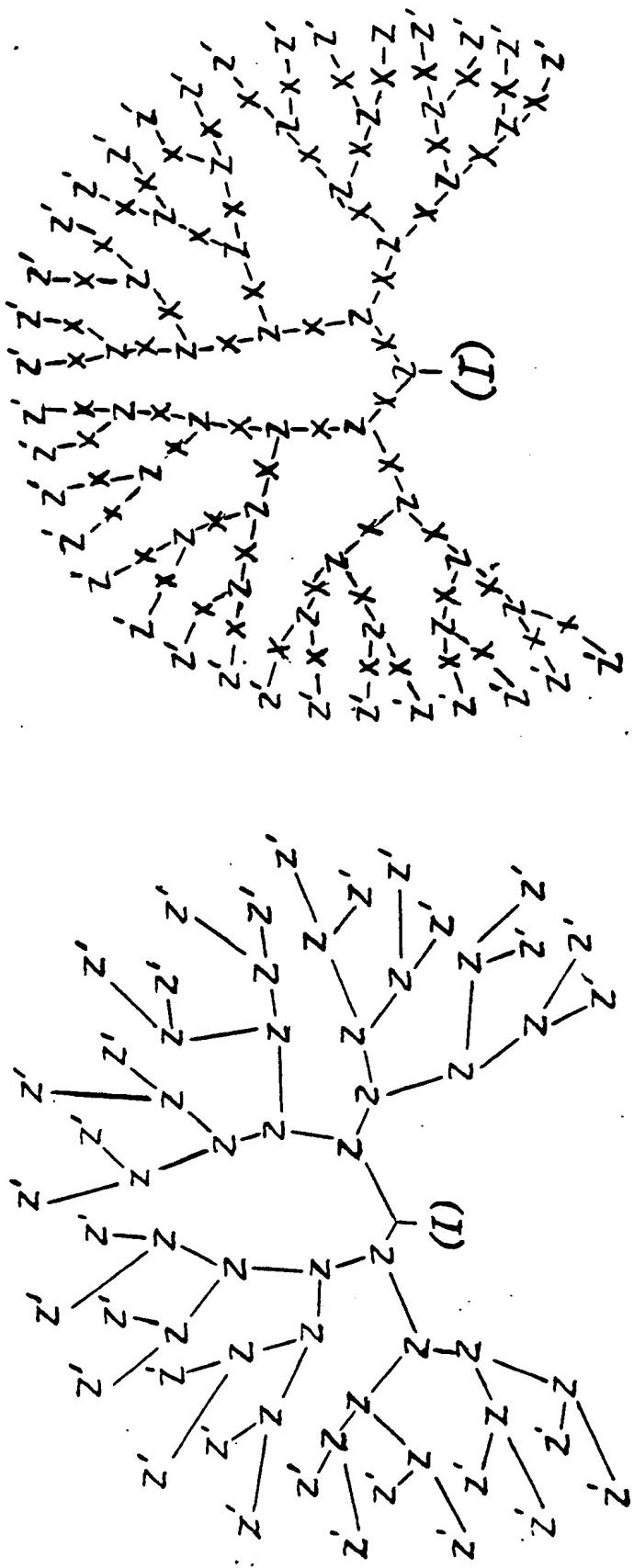
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FIG. 3



Generation of added PAMAM Starburst Dendrimer

RECORDED BY OPTICAL SCANNER



(A)

(B)

میوه

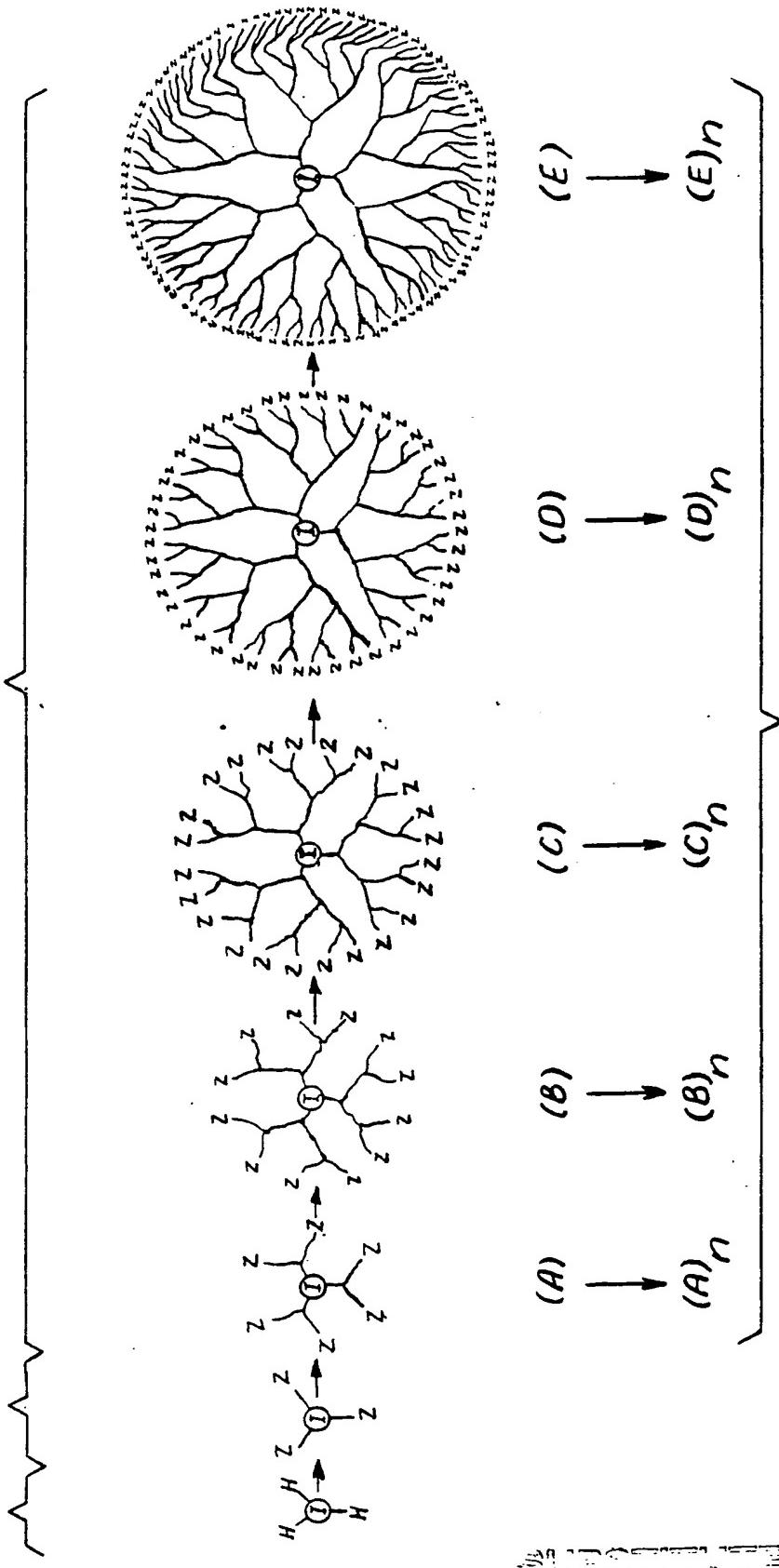


Fig. 1

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/02075

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

INT CL. 4 A 61K 49/02

US CL. 424/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
U.S.	424/1.1, 9 525/410, 416, 451, 418 528/310, 332, 350, 363, 397

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X Y	US, A, 4,558,120 PUBLISHED 10 December 1985 Tomalia (col. 12, lines 16-14)	1, 2, 6-13 3, 14, 15
P, A	US, A, 4,606,907 PUBLISHED 19 August 1986 Simon et al.	
T	US, A, 4,694,064 PUBLISHED 15 September 1987 Tomalia et al.	

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IV. CERTIFICATION

Date of the Actual Completion of the International Search ²⁰:

29 October 1987

Date of Mailing of this International Search Report ²¹:

25 NOV 1987

International Searching Authority ²²:

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Signature of Authorized Officer ²³

John S. Maples
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